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International application number: PCT/US05/001218

International filing date: 13 January 2005 (13.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/536,337
Filing date: 13 January 2004 (13.01.2004)

Date of receipt at the International Bureau: 20 May 2005 (20.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

1320389

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APPLICATION NUMBER: ***60/536,337***
FILING DATE: ***January 13, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/01218***

UNITED STATES PATENT AND TRADEMARK OFFICE
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 C.F.R. § 1.53(c).

	Docket Number	21101.0053U1	Type a Plus Sign (+) inside this box	+

INVENTOR(s)

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TITLE OF INVENTION (500 characters max)

COMPOSITIONS AND METHODS FOR MODULATING DHR96

CORRESPONDENCE ADDRESS

Customer Number 23859

ENCLOSED APPLICATION PARTS (*Check All That Apply)*

☒	Provisional Application Title Page	*Number of Pages*	[1]
☒	Specification (includes Description, Claims, & Abstract)	*Number of Pages*	[119]
☒	Drawing(s)	*Number of Sheets*	[8]
☒	Table	*Number of Sheets*	[1]
☒	Authorization to Treat Reply Requesting Extension of Time as Incorporating Petition for Extension of Time		
☒	Other (specify): Return Postcard		

METHOD PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (*Check One*)	
☒ Applicant claims small entity status. See 37 CFR § 1.27. ☐ A Credit Card Payment Form PTO-2038 is enclosed to cover the filing fees. ☐ A check or money order is enclosed to cover the filing fees. ☒ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 501977 . ☒ The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 14-0629.	**FILING FEE AMOUNT** $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

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Respectfully submitted,

Signature D E H 2 **Date** Janury 13, 2004

Typed or Printed Name: David E. Huizenga, Ph.D.

Registration No. 49,026

CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. § 1.10

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D E H 2 — Janury 13, 2004

David E. Huizenga — Date

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ATTORNEY DOCKET NO. 21101.0053U1
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Thummel *et al.*	Art Unit: Unassigned
Application No. Unassigned	Examiner: Unassigned
Filing Date: Concurrently	Confirmation No. Unassigned
For: COMPOSITIONS AND METHODS FOR MODULATING DHR96	

AUTHORIZATION TO TREAT REPLY REQUIRING EXTENSION OF TIME AS INCORPORATING PETITION FOR EXTENSION OF TIME

Mail Stop PROVISIONAL PATENT APPLICATION
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NEEDLE & ROSENBERG, P.C.
Customer Number 23859

Sir:

Pursuant to 37 C.F.R. § 1.136(a)(3), the Commissioner is hereby requested and authorized to treat any concurrent or future reply in the above-identified application, requiring a petition for an extension of time for its timely submission, as incorporating a petition for extension of time for the appropriate length of time.

ATTORNEY DOCKET NO. 21101.0053U1
PATENT

The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.

David E. Huizenga, Ph.D.
Registration No. 49,026

NEEDLE & ROSENBERG, P.C.
Customer No. 23859

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David E. Huizenga

January 13, 2004
Date

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Attorney Docket No. 21101.0053U1

UTILITY PATENT - PROVISIONAL FILING

PROVISIONAL APPLICATION FOR LETTERS PATENT

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Carl S. Thummel, a U.S. citizen, Kirst King-Jones, a British citizen, Michael Horner, a U.S. citizen, and Geanette Lam, a U.S. citizen, residing respectively at 2352 S. Lakeline Drive, Salt Lake City, UT 84109, 1416 Downington Ave, Salt Lake City, UT 84105, 1619 E. Wilson Ave., Salt Lake City, UT 84105, and 4984 S. Kalani Dr., Holliday, UT 84117, U.S.A.

have invented new and useful improvements in

COMPOSITIONS AND METHODS FOR MODULATING DHR96

for which the following is a specification.

COMPOSITIONS AND METHODS FOR MODULATING DHR96

I. BACKGROUND

1. The control of insects with toxins (pesticides) is one of the largest industries in the world. Insects have evolved many methods to deal with pesticides, most of which act through a xenobiotic detoxification pathway. The regulation of the xenobiotic pathway represents an attractive target for pesticides. Disclosed herein, DHR96, a Drosophila gene is shown to regulate the xenobiotic pathway, and inhibition of the DHR96 gene expression or activity decreases the ability of Drosophila to adapt to toxins, including pesticides, such as DDT.

II. SUMMARY

2. Disclosed are methods and compositions related to compositions and methods for regulating DHR96 and increasing the effect of existing any toxins to control insects are disclosed.

III. BRIEF DESCRIPTION OF THE DRAWINGS

3. The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments and together with the description illustrate the disclosed compositions and methods.

4. Figure 1 shows DHR96 is closely related to the PXR/CAR/VDR subfamily of xenobiotic receptors. An alignment using the programs PHYLIP and CLUSTALW is depicted of the DHR96, DAF-12, PXR, CAR, and NHR-8 nuclear receptors, showing the percent identical amino acids within either the DNA binding domain (green) or ligand binding domain (purple).

5. Figure 2 shows DHR96 is expressed in organs involved in nutrient absorption, metabolism, and excretion. Organs were dissected from wandering third instar larvae, fixed in 25% formaldehyde and stained with affinity-purified antibodies to detect DHR96 protein. In wild type larvae, nuclear DHR96 protein is detected in the fat body, in salivary glands and regions of the digestive tract including the gastric caece and the Malpighian tubules. Only background staining is detected in other tissues, including the imaginal discs and brain. No expression was detectable in fat bodies dissected from $DHR96^{E25}$ mutant larvae, demonstrating the specificity of the antibody stains.

6. Figure 3 shows a strategy for targeted mutagenesis of the DHR96 locus. Δ1 depicts the start methionine deletion and Δ2 depicts the deletion of the fourth exon/intron of *DHR96*. A transgene containing the targeting construct and the GFP marker was circularized by FLP

recombinase and subsequently cut with I-*SceI*. Homologous pairing between the targeting construct and the endogenous *DHR96* locus results in the generation of a tandem duplication by 'ends-in' recombination. To generate a single copy insertion, the tandem duplication was reduced by means of homologous recombination by inducing a DNA double stranded break with I–*CreI*.

7. Figure 4 shows DHR96 mutants are more sensitive than wild type flies to the pesticide DDT. Figure 4A shows a dose response curve. Twenty wild type or $DHR96^{E25}$ mutant flies were exposed to eight DDT concentrations, from 0.78 to 100 ng/µl, and then scored for survival 10 hours later. Figure 4B shows a time course. 20 wild type or $DHR96^{E25}$ mutant flies were treated with a high concentration of DDT (100 ng/µl) and assayed for survival every hour up to 10 hours. Each assay (A+B) was done in triplicate to determine the standard deviation as shown by the error bars.

8. Figure 5 shows an alignment of Drosophila nuclear hormone receptor DNA-binding domains. An alignment of the DNA-binding domains of known Drosophila nuclear hormone receptor superfamily members reveals two regions of conserved amino acids flanking a central unique region. The conserved amino acids were used to design PCR primers for amplifying fragments of Drosophila receptors: F3, F4, F5, R4, R5, R6 and R8. The unique region was used to design gene-specific oligonucleotide probes to eliminate previously identified family members from further study.

9. Figure 6 shows alignments of DNA-binding domain sequences. The DNA-binding domain sequence of each gene was used to search the PIR/Swiss Prot/GenBank databases. An alignment of each sequence with representative matches from the databases is presented. Shaded boxes indicate identity with the new protein sequence, and the percent identity is shown to the right of each sequence.

10. Figure 7 shows temporal profiles of DHR38, DHR78, and DHR96 transcription during the onset of metamorphosis. Northern blots containing RNA samples isolated from staged third instar larvae and prepupae collected at 2 hr intervals were probed to detect DHR38, DHR78, and DHR96 mRNAs. These blots have been used previously for detailed studies of 20E-regulated gene transcription ((Andres, A. J., Fletcher, J. C., Karim, F. D. & Thummel, C. S. (1993). Dev. Biol. 160, 388-404) One set of blots was sequentially stripped and hybridized with probes from each gene, in order to allow direct comparison of transcription patterns. The blots were also hybridized to detect rp49 mRNA, as a control for equal loading (data not shown)). Developmental times are shown at the top as hours after egg laying for third instar larval

development, and as hours after puparium formation for prepupal and pupal development. Landmark 20E-triggered developmental transitions are shown at the top.

11. Figure 8 shows a time course of DHR38, DHR78, and DHR96 transcription in cultured larval organs treated with 20E. Mass-isolated late third instar larval organs were treated with 5x10-7 M 20E for the times shown, as described (Thummel, C. S., Burtis, K. C. & Hogness, D. S. (1990). Cell 61, 101-111) Equal amounts of total RNA isolated from each time point were fractionated by formaldehyde agarose gel electrophoresis, transferred to a nylon membrane, and hybridized with probes to detect DHR38, DHR78, DHR96 and rp49 mRNA. One northern blot was sequentially stripped and hybridized with a probe from each gene, in order to allow direct comparison of transcription patterns. Detection of DHR38 transcripts required the use of an antisense RNA probe.

12. Figure 9 shows the DNA-binding specificities of DHR38, DHR78, and DHR96 protein. Each protein was overproduced in E. coli, purified, and tested for its ability to bind to eight oligonucleotides using electrophoretic mobility shift assays. The names of each oligonucleotide are shown at the top. In all cases, binding could be competed by the addition of an excess of the appropriate unlabelled oligonucleotide (data not shown).

13. Table 3 shows DHR96 regulates genes involved in detoxification. Control larvae and larvae carrying a *hs-DHR96* transgene were heat-shocked at 6 hours before pupariation and collected as white prepupae. Total RNA was extracted and purified to allow probe generation. Affymetrix microarray chips were hybridized with the probes and scanned. Raw data was analyzed with dCHIP, and filtering was performed in MS ACCESS. This table depicts the top 20 genes that are reduced in their expression in *hs-DHR96* transformants compared to control animals. The corresponding fold change is also shown. Red: Members of gene families known to be involved in detoxification in insects.

IV. DETAILED DESCRIPTION

14. Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods or specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

A. Definitions

15. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

16. Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed the "less than or equal to 10"as well as "greater than or equal to 10" is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point 15 are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15.

17. References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article, denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

18. A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

19. In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

20. "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

21. "Primers" are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art which do not interfere with the enzymatic manipulation.

22. "Probes" are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art.

23. Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

B. Compositions and methods

24. Higher organisms neutralize environmental toxins or xenobiotics through enzymes that include cytochrome p450 monooxygenases, glutathione transferases, carboxylesterases, and UDP-glucuronosyl transferases. In mammals, some of these detoxification enzymes are directly regulated by the nuclear receptors PXR and CAR, which in turn are activated by a broad spectrum of xenobiotics including prescription drugs, plant toxins and other contaminants. In contrast, there is little understanding of how similar xenobiotic responses might be controlled in insects. Herein it is shown that mutants in the DHR96 nuclear receptor of Drosophila are viable and fertile under standard laboratory conditions, as are flies that widely express double stranded *DHR96* RNA (RNAi) from a transgene. However, when exposed to a pesticide like DDT, mutant animals are less resistant to the insecticide challenge, dying more rapidly and at lower concentrations than control animals. Unlike many other nuclear receptors, widespread ectopic expression of DHR96 has no effect on the viability of larvae or flies, suggesting that activation of DHR96 is ligand-dependent. Disclosed herein, DHR96 is expressed in tissues that have been

associated with the detoxification process, including the gastric caeca, the major site of absorption in Diptera, and the fat body, the insect equivalent of the liver. Microarray studies disclosed herein show that overexpression of *DHR96* results in the downregulation of members of all four classes of the detoxification machinery, supporting the proposal that DHR96 functions as a xenobiotic regulator in *Drosophila*. These findings demonstrate how detoxification enzymes are activated in insects upon challenge with an insecticide. Given that this receptor has been highly conserved in the distant insect species, *Anopheles gambiae*, it is likely that it exerts a similar function in all insects. Also disclosed are methods for the identification of specific compounds or peptides that affect DHR96 activity and can act as effective synergists that, for example, enhance the lethality of pesticides for insect control.

25. Disclosed are mutants of the DHR96 gene which have reduced DHR96 activity in the xenobiotic pathway. These mutants can be used in a variety of methods for isolating new molecules that inhibit the xenobiotic pathway, by for example, being used as controls in methods that are testing the xenobiotic activity of a particular compound. The mutants can also be used as stock for production of other mutant flies. The mutants can also be used as seed genetic backgrounds to change a given population of flies to insecticide sensitive flies, by introducing the mutant backgrounds into the populations, through fly breeding.

26. Also disclosed are compositions which are capable of inhibiting DHR96 protein function or gene function, and which in turn inhibit the xenobiotic effect of the DHR96 protein. For example, disclosed are iRNA molecules which inhibit the function of DHR96 and inhibit the xenobiotic effect of DHR96.

27. Also disclosed are methods of inhibiting insect growth by administering an inhibitor of DHR96 to an insect, such as a fly.

28. Also disclosed are methods of identifying molecules that inhibit DHR96, and inhibit the xenobiotic activity in an insect, such as a fly, comprising for example, testing compounds for inhibition activity of DHR96 and/or inhibition of xenobiotic activity and, then for example, comparing the activity of these molecules to the disclosed inhibitors of DHR96, such as the mutants or the disclosed iRNA molecules.

1. The xenobiotic response

29. Virtually every organism faces a fundamental challenge when exposed to potentially harmful environmental substances called xenobiotics, which may include pharmaceuticals, plant toxins, pollutants, pesticides, hormones and fatty acids. Exposure to xenobiotics can occur either directly by physical contact, inhalation, or ingestion of nutrients or indirectly when an organism

generates toxic metabolites from less harmful precursors. The mechanisms by which toxic compounds are removed and/or neutralized fall into two broad categories. Usually as a result of extreme selective pressures, organisms may develop adaptive processes that are highly specific to a particular substance, as can be observed in many insect species that become resistant to pesticides (Wilson, T. G. (2001). Annu Rev Entomol 46, 545-571) or that have evolved the ability to utilize hazardous plant species as a food source (Danielson, P. B. et al. (1997). Proc Natl Acad Sci U S A 94, 10797-10802; Fogleman, J. C. (2000). Chem Biol Interact 125, 93-105.). In contrast to this highly specific response, all metazoan species appear to have a general machinery that allows the efficient detoxification of a vast range of chemicals. The general detoxification mechanisms display a surprising flexibility, which is mainly achieved by two factors. First, at least three enzyme classes comprising more than 160 proteins in the mosquito and the fruit fly are responsible for metabolizing lipophilic toxins into less harmful substances (Ranson, H., et al. (2002). Science 298, 179-181). Second, some enzymes appear to have an immense range of substrate specificity. For instance, Cyp3A4, a member of the cytochrome p450 monooxygenase family, is capable of neutralizing an estimated 50% of all existing prescription drugs (Maurel, P. (1996). (Boca Raton, CRC Press), pp. 241-270). Cytochrome p450 enzymes are often referred to as phase I enzymes, because they catalyze the first step in the detoxification process by adding oxygen groups to lipophilic chemicals, thus resulting in more water-soluble compounds, which in turn facilitates efficient excretion. Other enzyme families like glutathione transferases, carboxylesterases and UDP-glucuronosyl transferases are classified as phase II enzymes, as their role is to catalyze subsequent detoxification steps.

30. In insects, pesticide resistance is most often the result of mutations that affect the general detoxification pathway. For example, the overexpression of a single gene, *Cyp6g1*, a member of the cytochrome p450 family, is sufficient to confer DDT resistance in *Drosophila melanogaster* (Daborn, P. B. et al. (2002), Science 297, 2253-2256). The same study demonstrated that *Cyp6g1* is hypertranscribed in over 20 DDT-resistant *Drosophila* strains of worldwide origin, but further analysis suggested that this finding could be traced back to a single event, since all alleles harbor the same *Accord* transposon in their 5' regulatory region.

31. In the past decade considerable progress in the field has revealed the mechanisms that allows an organism to sense a wide range of toxic substances and to understand how xenobiotic sensing translates into the induction of highly specific sets of detoxifying enzymes. It quickly became apparent that certain members of the so-called nuclear receptor superfamily are the central players in this process. Nuclear receptors are ligand-activated transcription factors that

play important roles in diverse physiological processes such as cell growth and differentiation, embryonic development, and cholesterol metabolism (Francis, G. A. et al. (2003) Annu Rev Physiol 65, 261-311; Mangelsdorf, D. J.,et al. (1995). Cell 83, 835-839; Tontonoz, P., and Mangelsdorf, D. J. (2003). Mol Endocrinol 17, 985-993) Of the 48 nuclear receptors encoded by the human genome ~26 have identified ligands (Kliewer, S. A. (2003) J Nutr 133, 2444S-2447S), but only three have been associated with xenobiotic activity, namely PXR, CAR and VDR (Maglich, J. M., et al. (2002) Mol Pharmacol 62, 638-646; Makishima, M., et al. (2002). Science 296, 1313-1316). These three closely related receptors are not only able to sense and bind lipophilic xenobiotic substances directly, but once activated by such a ligand, they can regulate the expression of enzymes that will neutralize the very compound that had activated these nuclear receptors in the first place, thus creating feedback loop. Disclosed is an analogous mechanism that exists in the fruit fly, *Drosophila melanogaster*. The disclosed mechanism involves an insect nuclear receptor, the Drosophila DHR96 nuclear receptor.

(1) Nuclear receptors

32. Members of the nuclear receptor superfamily have been one of the most productive targets for drug development by the pharmaceutical industry. Efforts along these lines have resulted in drugs that have had a major impact on human health, including cancer treatments, fertility control, and cholesterol reduction. Nuclear receptors are ligand-activated transcription factors, but can have many regulatory functions aside from this ligand activated function. Nuclear receptors have been organized in a phylogeny-based nomenclature (Nuclear Receptors Nomenclature Committee, (1999) Cell 97, 1-3.) of the form NR*xyz*, where *x* is the sub-family, *y* is the group and *z* the gene. For a review see, Robinson-Rechavi, M., et al., Journal of Cell Science, Cell Science at a Glance, 116(4):585-586 and poster insert, (2003), which is herein incorporated by reference at least for material related to nuclear receptors).

33. Nuclear receptors lend themselves to drug intervention because their activity can be modulated by small lipophilic compounds that can be easily delivered to animals in a stable format. Compounds can be developed that either constitutively activate their cognate receptor, called agonists, or constitutively inactivate the receptor, called antagonists. The use of these compounds in animals provides a means of tightly regulating nuclear receptor activity *in vivo*, with resultant effects on growth and development.

34. Surprisingly, no similar effort has been made by the agricultural industry to target insect nuclear receptors as a means of pest control. This is largely because the mechanism of action of most insect nuclear receptors has remained undefined. Disclosed herein it was shown

that an insect nuclear receptor, encoded by *DHR96*, is required for resistance to toxic compounds in *Drosophila*. Also disclosed are molecules that inhibit the DHR96 function and that inhibiting the function of DHR96 makes DHR96 have decreased resistance to pesticides and toxins. Also disclosed are methods utilizing DHR96 to identify compounds that modulate its function, such as inhibit its function. Molecules that inhibit DHR96 render the insect more susceptible and sensitive to pesticides.

35. The *Drosophila* genome encodes 18 nuclear receptors that have a classical DNA-binding and ligand-binding domain and, of those, just two have identified ligands. In the nematode *C. elegans*, it was shown that a mutation in the nuclear receptor *nhr-8* gene causes a reduced resistance to colchicine and chloroquine, suggesting that this gene is involved in the xenobiotic pathway (Lindblom, T. H., et al. (2001). Curr Biol 11, 864-868, which is herein incorporated by reference at least for material related to nuclear receptors and their activity, and for material related to NHR8). Disclosed herein *DHR96* mutants are viable under normal conditions, but exhibit a significantly lower resistance to DDT when compared to wild type flies. Additionally, microarray analysis of animals that overexpress DHR96 indicate that this nuclear receptor regulates genes which primarily encode detoxification enzymes.

36. Disclosed herein insecticide function in insects can be reviewed from a different perspective. Disclosed are methods for identifying DHR96 antagonists and agonists. Also disclosed are methods related to the identification of the DHR96 target gene network. Also disclosed is a class of pesticides that targets the regulatory pathways that control the detoxification machinery.

(a) Classes of nuclear receptors

37. Retinoid, vitamin D, steroid, and thyroid hormones are small hydrophobic ligands that initiate a diverse array of developmental and metabolic responses. The receptors that mediate these responses form the basis of the nuclear hormone receptor superfamily (see Tsai, M.-J. & O'Malley, B. W. (1994). *Annu. Rev. Biochem.* 63, 451-486, for a review). This family is defined by a characteristic protein domain structure including a conserved DNA-binding domain and a ligand binding/dimerization domain. Members of this superfamily can be divided into three classes based on their ligand-binding and DNA-binding properties. Steroid receptors, including the estrogen and glucocorticoid receptors, form homodimers that bind to an inverted repeat of 6 bp consensus half-sites (Tsai, M.-J. & O'Malley, B. W. (1994). *Annu. Rev. Biochem.* 63, 451-486, Gronemeyer, H. (1992). *FASEB J.* 6, 2524-2529). The second class includes the retinoid receptors, RAR and RXR, as well as receptors for thyroid hormone and vitamin D.

These receptors can bind to direct repeats of AGGTCA half-sites as homodimers or heterodimers (Stunnenberg, H. G. (1993). *BioEssays* 15, 309-315). The third and largest class are referred to as orphan receptors since their potential ligands are unknown. At least some of these receptors, including Rev-Erb and NGFI-B, can bind to a single AGGTCA half-site (Harding, H. P., & Lazar, M. A. (1993). *Mol. Cell. Biol.* 13, 3113-3121; Wilson, T. E., et al., (1993). *Mol. Cell. Bio.* 13, 5794-5804). Although extensive studies have provided significant insights into the mechanisms by which nuclear hormone receptors regulate the transcription of target genes, we still know little about how these changes in gene expression result in specific and diverse developmental responses.

(b) Drosophila nuclear receptors

38. There are 18 canonical nuclear receptor genes in the complete genome of the fly *Drosophila melanogaster* (Adams et al., (2000) *Science* 287, 2185-2195, which is herein incorporated by reference at least for material related to nuclear receptors). The 18 members of the nuclear hormone receptor superfamily identified in *Drosophila* are: *EcR, usp, tll* (Pignoni, F., et al., (1990). *Cell* 62, 151-163), *svp* (Mlodzik, M., et al., (1990). *Cell* 60, 211-224), *dHNF-4* (Zhong, W., et al., (1993). *EMBO J* 12, 537-544), *E75* (Segraves, W. A. & Hogness, D. S. (1990). *Genes Dev.* 4, 204-219), *E78* (Stone, B. L. & Thummel, C. S. (1993). *Cell* 75, 307-320), *FTZ-F1* (Lavorgna, G., et al., (1991). *Science* 252, 848-851), *DHR3* (Koelle, M. R., et al., (1992). *Proc. Natl. Acad. Sci. USA* 89, 6167-6171), DHR4 (Weller J, Sun GC, Zhou B, Lan Q, Hiruma K, Riddiford LM. Isolation and developmental expression of two nuclear receptors, MHR4 and betaFTZ-F1, in the tobacco hornworm, *Manduca sexta*. Insect Biochem Mol Biol. 2001 Jun 22;31(8):827-37.; King-Jones, K. Charles, J.-P., & C.S. Thummel, The DHR4 orphan nuclear receptor is required for Drosophila growth and metamorphosis, manuscript in prep; Adams et al., (2000) *Science* 287, 2185-2195) and *DHR39* (Ohno, C. K. & Petkovich, M. (1992). *Mech. Dev.* 40, 13-24; Ayer, S., et al., (1993). *Nuc. Acids Res.* 21, 1619-1627), *DHR38, DHR78* (Fisk and Thummel, (1995), PNAS, Proc Natl Acad Sci U S A. 1995 Nov 7;92(23):10604-8), *DHR83* (King-Jones, K. and C.S. Thummel (2003) Drosophila nuclear receptors. In"Handbook of Cell Signaling," Vol. 3, (Bradshaw, R. and Dennis, E., eds.),Academic Press, New York, pp. 69-73; Adams et al., (2000) *Science* 287, 2185-2195), *DHR96* (Fisk and Thummel, 1993), *dsf* (Finley, K. D., et al. (1998). "dissatisfaction encodes a Tailless-like nuclear receptor expressed in a subset of CNS neurons controlling Drosophila sexual behavior." Neuron 21, 1363-1374), *dERR* (King-Jones, K. and C.S. Thummel (2003) Drosophila nuclear receptors. In"Handbook of Cell Signaling," Vol. 3, (Bradshaw, R. and

Dennis, E., eds.),Academic Press, New York, pp. 69-73; Adams et al., (2000) *Science* 287, 2185-2195), and *dFAX-1* (King-Jones, K. and C.S. Thummel (2003) Drosophila nuclear receptors. In"Handbook of Cell Signaling," Vol. 3, (Bradshaw, R. and Dennis, E., eds.),Academic Press, New York, pp. 69-73; Adams et al., (2000) *Science* 287, 2185-2195) At least seven of these genes appear to contribute to the 20E regulatory hierarchies that direct the onset of metamorphosis – *E75*, *E78*, *βFTZ-F1*, *DHR3*, *DHR39*, *EcR*, and *usp* (Richards, G. (1992). *Current Biology* 2, 657-659; Horner, M., et al., (1995). *Dev. Biol.* 168, 490-502; Woodard, C. T., et al., (1994). *Cell* 79, 607-615).

39. Table 5 provides a list of Drosophila nuclear receptors.

40. Table 5

probe set	CG	CT	Accession	Description	SEQ ID NO
144004_at	CG16902	CT37504	FBgn0023546	sym=Hr4 orEG:133E12.2 /name= DHR4	SEQ ID NO:1
154699_at	CG4059	CT13432	FBgn0001078	sym=ftz-f1 /name=ftz transcription factor 1	SEQ ID NO:3
143123_at	CG11823	CT11367	FBgn0000448	sym=Hr46 or DHR3 /name=Hormone receptor-like in 46	SEQ ID NO: 5
152580_at	CG11783	CT33046	FBgn0015240	sym=Hr96 or DHR96/name=Hormone receptor-like in 96	SEQ ID NO: 7
143535_at	CG9310	CT40906	FBgn0004914	sym=Hnf4 /name=Hepatocyte nuclear factor 4	SEQ ID NO: 9
143768_at	CG1864	CT5732	FBgn0014859	sym=Hr38 or DHR38 /name=Hormone receptor-like in 38	SEQ ID NO: 11
149398_at	CG10296	CT28911	FBgn0037436	sym=CG10296 or DHR83 /name=Hr83	SEQ ID NO: 13
143372_at	CG11502	CT12919	FBgn0003651	sym=svp /name=seven up /prod=nuclear receptor NR2F3	SEQ ID NO: 15
143379_at	CG1378	CT3134	FBgn0003720	sym=tll /name=tailless /prod=nuclear receptor NR2E2	SEQ ID NO: 17
143805_at	CG9019	CT25922	FBgn0015381	sym=dsf /name=dissatisfaction /prod= /func=receptor	SEQ ID NO: 19
147244_at	CG16801	CT37351	FBgn0034012	sym=CG16801 /name=FAX-1 /prod=nuclear hormone receptor-like	SEQ ID NO: 21
153072_at	CG7404	CT22787	FBgn0035849	sym=CG7404 /name=ERR /prod= /func=steroid hormone receptor	SEQ ID NO: 23
152160_at	CG7199	CT22217	FBgn0015239	sym=Hr78 or DHR78/name=Hormone-receptor-like in 78	SEQ ID NO: 25
153675_at	CG4380	CT14272	FBgn0003964	sym=usp /name=ultraspiracle /prod=nuclear receptor NR2B4	SEQ ID NO: 27

153197_at	CG8127	CT24290	FBgn0000568	sym=Eip75B or E75/name=Ecdysone-induced protein 75B	SEQ ID NO: 29
143525_at	CG18023	CT40336	FBgn0004865	sym=Eip78C or E78/name=Ecdysone-induced protein 78C	SEQ ID NO: 31
154377_at	CG1765	CT5200	FBgn0000546	sym=EcR /name=Ecdysone receptor /prod=ecdysone receptor	SEQ ID NO: 33
155094_at	CG8676	CT5296	FBgn0010229	sym=EcR /name=Ecdysone receptor /prod=ecdysone receptor	SEQ ID NO: 35

41.

42. While there are 18 nuclear receptors in flies, there are 48 in humans (Robinson-Rechavi et al., (2001) *Trends Genet* 17, 554-556), 49 in the mouse with the addition of FXRβ, (Robinson-Rechavi and Laudet, 2003, Methods Enzymol. 2003;364:95-118) and more than 270 genes in the nematode worm *Caenorhabditis elegans* (Sluder et al., (1999). Genome Research 9, 103-120.

(c) Role of 20-hydroxyecdysone(20E) in Drosophila

43. 20E is involved in the metamorphosis of the fruit fly, *Drosophila melanogaster* through steroid hormone receptors. A high titer 20E pulse at the end of third instar larval development triggers puparium formation, followed 10 hrs later by an 20E pulse that triggers head eversion and the onset of pupal development (Pak, M. D. & Gilbert, L. I. (1987). *J. Liq. Chrom.* **10,** 2591-2611; Richards, G. (1981). *Mol. Cell. Endocrin.* **21,** 181-197). The 20E receptor is encoded by two members of the nuclear hormone receptor superfamily, *EcR* (Koelle, M. R., et al., (1991). *Cell* **67,** 59-77) and *usp* (Henrich, V. C., et al., (1990). *Nuc. Acids Res.* **18,** 4143-4148; Shea, M. J., et al., (1990). *Genes Dev.* **4,** 1128-1140; Oro, A. E., et al., (1990). *Nature* **347,** 298-301). Usp is most closely related to the vertebrate RXR family and can heterodimerize with vertebrate thyroid and vitamin D receptors, as well as with EcR (Yao, T., et al., (1992). *Cell* **71,** 63-72; Thomas, H. E., et al., (1993). *Nature* **362,** 471-475; Yao, T., et al., (1993). *Nature* **366,** 476-479; Koelle, M. R. (1992) Ph.D. thesis, Stanford University). The ability of RXRs to function as promiscuous heterodimerization partners combined with the sequence similarity of many receptor binding sites raises the possibility that other members of the superfamily may function in transducing 20E signals, either by interacting directly with EcR and/or Usp, or by competing for receptor binding sites (Richards, G. (1992). *Current Biology* **2,** 657-659).

(d) General structure of nuclear receptors

44. There are a number of domains in a nuclear receptor. From the N terminus to the C terminus there is the A/B domain, followed by a DNA binding domain (DBD, C), which contains the DNA sequence recognition domain called the P-box, which is followed by a less conserved region, D, which acts as a flexible hinge between the DBD and the ligand binding domain (LBD, E) and the D domain typically contains the nuclear localization signal, but this may overlap with the C domain, and finally some nuclear receptors contain a C-terminal F domain whose function is unknown.

45. The A/B domain and N terminal region in general is highly variable and can range in size from less than about 50 amino acids to more than about 500 amino acids. The A/B domain typically contains the transactivation domains which typically include at least one constitutively active domain, the AF-1 domain, and than typically one or more autonomous activation domains which can be regulated or not, called AD domains.

46. The DBD is typically the most conserved region. It contains the P-box, a six amino acid region that confers specificity for binding to particular target sites in the DNA. The P-box for DHR96 is ESCKA. An example of DHR96 is shown in SEQ ID NO:7. The DBD is also typically the site of homo- and hetero- dimerization. The 3D structure of the DBD shows that it contains contains two highly conserved zinc- fingers – C-X2-C-X13-C-X2-C and CX5- C-X9-C-X2-C – the four cysteines of each finger chelating one Zn^{2+} ion.

47. The LBD is typically the largest domain and is only moderately conserved, but the secondary structure is often conserved and contains 12 α-helixes. Many functions are associated with the E domain, including the AF-2 transactivation function, a strong dimerization interface, another NLS, and often a repression function. Typically the functions are ligand regulated.

(e) Dimerization of nuclear receptors.

48. Dimerization of nuclear receptors is very important to their function. The dimerization domains typically reside in the DBD and LBD. Many nuclear receptors heterodimerize with RXRs (USP in arthropods), such as DHR38 (NR4A4), NGFIB (NR4A1), NURR1 (NR4A2), NOR1 (NR4A3), LXR and FXR subfamilies (LXRα, (NR1H3), LXRβ (NR1H2, HO), ECR (NR1H1), FXRα (NR1H4, HO), FXRβ (NR1H5, HO), the CAR1 and VDR subfamilies including, CAR1 (NR1I3), PXR (NR1I2), VDR (NR1L1) (NR1J1), the PPAR subfamily including, PPARγ (NR1C3), PPARα (NR1C1), AND PPARβ (NR1C2), the RAR subfamily including RARβ (NR1B2), RARα (NR1B1), and RARγ (NR1B3), and TRα (NR1A1), and TRβ (NR1A2), and possibly COUP-TF and FXRβ (for a review, see Robinson-Rechavi M,

Escriva Garcia H, Laudet V., J Cell Sci. 2003 Feb 15;116(Pt 4):585-6). DHR96 can also be found to dimerize with any other receptor, such as USB, or itself.

(f) Ligands for nuclear receptors

49. The superfamily includes receptors for many different types of molecules. For example, nuclear receptors bind hydrophobic molecules such as steroid hormones, such as estrogens, glucocorticoids, progesterone, mineralocorticoids, androgens, vitamin D3, ecdysone, oxysterols and bile acids. Certain nuclear receptors also bind retinoic acids , such as all-trans and 9-cis isoforms, thyroid hormones, fatty acids, leukotrienes and prostaglandins (Escriva et al., 2000, Bioessays 22, 717-727 and Robinson-Rechavi M, Escriva Garcia H, Laudet V., J Cell Sci. 2003 Feb 15;116(Pt 4):585-6).

(g) How nuclear receptors function

50. Nuclear receptors typically act in a stepwise fashion that starts with repression, moves to a state of derepression, and ends with transcription activation. (reviewed by Robinson-Rechavi M, Escriva Garcia H, Laudet V., J Cell Sci. 2003 Feb 15;116(Pt 4):585-6).

51. Repression typically occurs with corepressors, such as the histone deacetylase activity (HDAC) (for example, the apo-nuclear receptor). Usually ligand binding results in derepression, caused by the disassociation of the receptor from the corepressors. Also ligand binding typically causes the recruitment of coactivators, such as histone acetyltransferase (HAT) activity, which causes chromatin decondensation, which is believed to be necessary but not sufficient for activation of the target gene. After the HAT complex dissociates, typically a second coactivator complex is assembled (TRAP/DRIP/ARC), which is able to establish contact with the basal transcription machinery, and thus results in transcription activation of the target gene, but many other transcription co-activators can be associated with the nuclear receptor and these coactivators can provide activation discrimination. This general scheme does not apply for all nuclear receptors, as for example, some nuclear receptors can activate without ligand and some may bind DNA without ligand and some may repress with or without ligand.

(2) DHR96 gene

52. *DHR96* maps to 96B12-14 in the polytene chromosomes of *Drosophila*. The DHR96 gene was cloned and sequenced and its sequence is set forth in SEQ ID NO:1. (Fisk and Thummel (1995) Proc. Natl. Acad. Sci USA, 92: 10604-10608, herein incorporated by reference at least for material related to the DHR96 gene and its sequence including the specific sequence).

53. *DHR96* is highly conserved in *Anopheles gambiae*, a distant (~ 250 M years) dipteran species (see Table 4). Similarly, many other *Drosophila* nuclear receptors are conserved in even

more distant insects and, when examined, their regulatory functions appear to be conserved as well (Swevers L, Iatrou K. The ecdysone regulatory cascade and ovarian development in lepidopteran insects: insights from the silkmoth paradigm. Insect Biochem Mol Biol. 2003 Dec;33(12):1285-97; Riddiford LM, Hiruma K, Zhou X, Nelson CA. Insights into the molecular basis of the hormonal control of molting and metamorphosis from Manduca sexta and Drosophila melanogaster. Insect Biochem Mol Biol. 2003 Dec;33(12):1327-38). This is consistent with the role of detoxification via *DHR96* being conserved through evolution. Thus, inactivation of *DHR96* function in known insect pests provides a novel mode of intervention. It is understood that DHR96 homologs in other insects, insect orders, insect families and other insect specifies are considered disclosed and that they function in a manner similar to DHR96 in Drosophila. There is significant homology within the order Diptera and within the class of insects in general for nuclear receptors, and there is shown in Table 4, that there is a high degree of homology between DHR96 in other insects, such as the mosquito.

54. Disclosed are DHR96 variants that have at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or homology as discussed herein in to the LBD of DHR96, DBD of DHR96, or full length DHR96, or of fragments of DHR96, functional or otherwise.

55. Among the C. elegans receptors, DHR96 is most similar to DAF-12, which is a gene involved in dauer larva formation in C. elegans (68% identity DBD; 29% identity LBD). The match with NHR-8 in C. elegans is weaker (60%; 25%). This is consistent with DHR96 having a role similar to DAF-12. DAF-12 reads signals from TGFbeta and insulin and decides when the worm should enter diapause to survive difficult conditions. Diapause is similar to pupal stages in many ways (indeed many insects diapause during metamorphosis). Disclosed herein, mutants of DHR96 did not have any effects on metamorphosis – and they survived. Thus it was expected that DHR96 would have a function similar to DAF-12. DAF-12 is a gene involved in dauer larva formation in C. elegans. DAF-12 reads signals from TGFbeta and insulin and decides when the worm should enter diapause to survive difficult conditions. Diapause is similar to pupal stages in many ways (indeed many insects diapause during metamorphosis). However, as disclosed herein, mutants of DHR96 did not have any effects on metamorphosis – as they survived.

56. Disclosed are systems that assay for effects of drugs that alter DHR96 – and thus one can assay for effects on target gene transcription and relate that expression to the ability of an animal, such as an insect, to resist toxins.

57. Table 4

species	DBD amino acids 7-72 identity	p-box	LBD amino acids 501-723 identity
anopholes gambiae	86% %	same	65% %
c.elegans daf-12	69%	same	26%
strongyloides stercoralis-parasitic worm	67%	different	27%
c.elegans nhr-48	66% %	same	
VDR-zebrafish	65%	different	27%
VDR-bastard halibut	63%	different	27%
mouse vdr	62%	different	23%
human vdr	62%	different	24%
c.elegans nhr-8	60%	same	25%
mouse pxr	59%	different	23%
human pxr	59%	different	22%
human car	56%	different	19%
AamEcRA1-tick	54%	different	
ecdysone receptor-locusta migratoris-locust	53%	different	
ecdysone receptor-calliphor vicina-insect	53%	different	
EcR- tenebrio molitor-yellow mealworm	53%	different	
EcR- d. melanogaster	51%	different	
EcR- aedes albopictus-mosquito	51%	different	
mouse car	51%	different	20%

58.

59. Table 4 shows the percent identical amino acids within the DNA binding domain and ligand binding domain for DHR96 and the best matches in the public databases (Genbank). Shown is the mosquito DHR96 gene, and it is the orthologous receptor in mosquito. (anopholes gambiae) (85% and 65% identity - very high). Also listed is whether the sequence within the P box, is either the same as DHR96 or different. This sequence directs the DNA binding specificity of the receptor. DHR96 DNA binding is predicted to be similar to that of all three nematode homologs (daf-12, nhr-48 and nhr-8), but none of the vertebrate ones.

60. In certain embodiments homologs of DHR96 in other insect species can have at least 50% identity in the DBD and 25% identity in the LBD.

61. An alignment of the *Drosophila* nuclear hormone receptor DNA-binding domains reveals a central region of 8-9 unique amino acids flanked by highly conserved regions that each contain a C_2C_2 zinc finger (Fig. 5).

62. The DNA-binding domain of DHR96 is 64% identical to the human vitamin D receptor and 52% identical to EcR (Fig. 6C). The DHR96 ligand binding domain (amino acids 501 - 723) is most similar to that of thyroid hormone receptor, with 23% identity.

63. *DHR96* encodes a 2.8 kb transcript that is expressed throughout third instar larval and prepupal development, with distinct increases in abundance at 106 hrs after egg laying (Fig. 7). The temporal patterns of *DHR96* transcription most closely resemble those of the genes encoding the 20E receptor. *EcR* and *usp* mRNAs can be detected throughout third instar larval and prepupal development (Andres, A. J., et al., (1993). *Dev. Biol.* **160,** 388-404; 36; Henrich, V. C., et al., (1994). *Dev. Biol.* **165,** 38-52).

64. The *hsp27* EcRE is the only oligonucleotide bound by DHR96, albeit it a weak interaction (Fig. 9). The EcRE consists of a palindromic arrangement of the imperfect half-sites AGtgCA and gGtTCA. DHR78 and DHR96 recognize distinct sequences that can also be bound by the EcR/Usp heterodimer (Horner, M., et al., (1995). *Dev. Biol.* **168,** 490-502). These distinct binding specificities are consistent with the P-box sequences of the DHR78 and DHR96 proteins. The DHR78 P-box, EGCKG, like that of DHR38, directs binding to an AGGTCA half-site sequence (Tsai, M.-J. & O'Malley, B. W. (1994). *Annu. Rev. Biochem.* **63,** 451-486). In contrast, DHR96 contains a unique P-box sequence that is only present in its three *C. elegans* homologs (see Table 4 above) – ESCKA The binding of the hsp27 EcRE by DHR96 is very weak. An optimal DNA binding site can be identified by further experimentation.

65. It will be of interest to determine whether DHR78 or DHR96 can heterodimerize with EcR, Usp, or any of the *Drosophila* orphan receptors.

(a) DHR96 functions in the xenobiotic pathway

66. Several lines of evidence support the conclusion that *DHR96* acts in a xenobiotic pathway. First, the protein is selectively expressed in tissues involved in nutrient absorption (gastric cacae), metabolism (fat body), and excretion (Malpighian tubules) – tissues that should play a primary role in detoxification and elimination of both endobiotic and xenobiotic compounds. Second, *DHR96* mutants, like null mutants in the mouse PXR and CAR xenobiotic nuclear receptors, are viable and fertile, indicating no critical role in normal development. Third, *DHR96* mutants are more sensitive to the pesticide DDT. Fourth, the most highly repressed genes in response to *DHR96* overexpression comprise members of all four classes of insect detoxifying genes.

67. The effect of the mutants can be confirmed by the expression of wild type *DHR96* (from a heat-inducible *DHR96* transgene, for example) in a homozygous mutant background, and

test for DDT sensitivity. This experiment should rescue the sensitivity back to wild type levels. In addition, *DHR96* function was reduced by RNAi and this results in levels of DDT sensitivity that are similar to those of *DHR96* mutants.

68. The decreased resistance to DDT in *DHR96* mutants can be confirmed as related to the inability to neutralize toxins rather than a general lack of fitness by demonstrating that sensitivity of *DHR96* mutants occurs for toxic compounds. It can also be confirmed by showing that detoxifying genes fail to be induced in *DHR96* mutants treated with toxic compounds, by for example, microarray analysis, with the mutants in the presence or absence of a toxin. These results could be compared to the microarray data disclosed herein. Two toxins that could be used for this are DDT and phenobarbital because the latter was shown to induce a number of cytochrome P450 genes in *Drosophila* (Danielson, P. B. et al. (1998) Mol Gen Genet 259, 54-59).

69. The expression of DHR96 and its activation level can be assayed to determine if it is directly activated by toxic compounds, similar to the ability of xenobiotics to bind to human PXR xenobiotic nuclear receptor. This can be done using transformed *Drosophila* that express a fusion of the yeast GAL4 DNA binding domain to the ligand binding domain of DHR96. When combined with a GAL4-dependent *lacZ* reporter gene, the expression of β-galactosidase will only occur when the DHR96 ligand binding domain is in an active conformation. This could be caused by a direct interaction between DHR96 and the toxin. Larval organs that carry these constructs can be cultured in the presence of various xenobiotic inducers, testing for induction of *lacZ* reporter gene activity. Furthermore, target gene promoters can be identified which can also demonstrate a direct interaction between DHR96 and the expression of a detoxifying enzyme.

70. In the disclosed microarray study, *DHR96* was overexpressed and it was found that this resulted in repression of a significant number of members of the major detoxification gene families. Repression of cuticle proteins was also observed, consistent with a role for cuticle formation in inhibiting pesticide toxicity (Wilson, T. G. (2001). Annu Rev Entomol 46, 545-571). The observation that these target genes are repressed suggests that DHR96 might function as a repressor in the absence of ligand. This is consistent with the action of other nuclear receptors, for example, both Endocrine receptor (EcR) and thyroid receptor (TR) are known to function in this manner. Very strict filtering criteria were used in the disclosed microarray experiments further strengthening the results.

71. The microarray studies allow the identification of the direct targets of DHR96. This will allow the identification of the genetic hierarchy that is regulated by this nuclear receptor.

Once target genes have been identified, it will be possible to construct reporter genes that are inducible by endogenous DHR96. Such a system can then be utilized to screen for drugs or combinations of drugs that activate or repress these reporter genes, in both a wild type and *DHR96* mutant background. This can further confirm that *DHR96* can directly regulate the expression of detoxifying genes. This system would also provide a direct readout of DHR96 activity that would be useful for further studies of *DHR96* function and for the development of appropriate inhibitors of DHR96 function. The mutants of DHR96 can be used to identify and confirm other factors that can act as xenobiotic receptors in insects, and test whether these act in a partially redundant manner with *DHR96*.

(3) Mutants of the DHR96 gene

72. Various DHR96 mutant alleles were made. A series of studies to characterize the *DHR96* mutant alleles were performed. These included Southern, Northern and Western blotting, tissue stains, sequencing of PCR products, and genetic mapping to validate the mutations in the different *DHR96* alleles. Validation of these alleles was particularly important because flies homozygous for *DHR96* mutations are viable and fertile. At least one of the alleles generated, $DHR96^{16A}$, is a protein null, because the translation start site was deleted and no protein was detectable in Western blots or tissue stains of homozygous mutant animals.

73. Gene targeting (Rong, Y. S., and Golic, K. G. (2000). Science 288, 2013-2018) was used to generate mutations in *DHR96* because no deficiencies or P elements were known in this region of the genome. (see Example 1). Using these methods any mutations of the DHR96 gene can be made, such as mutations at or around the start site; mutations at or around the splice sites; mutations which prevent or render inactive complete or partial exon sequences; mutations which render inactive or remove the complete or partial DBD or LBD or any of the domains of DHR96 discussed herein that it contains as a nuclear receptor.

74. The DHR96 gene resides on the third chromosome. When mutations are made in certain embodiments the mutations of the DHR96 gene are made such that there is only a single copy of the mutant and no copies of the wildtype gene in the insect, such as the fly. This is done, for example, by using vectors for the mutation generation, which have sites built in that allow for recombination and excision of the site, and fly stocks containing a single copy can be selected. (see for example, Rong, Y. et al., (2002) Genes Dev *16*, 1568-1581).

75. Disclosed are null mutants of the DHR96 gene. A null mutant is defined herein as a mutant that lacks functional DHR96 protein product.

76. A null mutant disclosed herein is *DHR96*^{16A} which is mutant having two specific deletions, one removing the start codon for translation and the second removing intron/exon 4, deleting a critical portion of the LBD.

77. Another null mutant disclosed herein is the mutant *DHR96*^{E25} which carries a tandem duplication of the *DHR96* gene in place of the single wild type copy. One of these mutant *DHR96* genes is identical to the *DHR96*^{16A} allele described above, missing both the start codon and intron/exon 4. The other mutant *DHR96* gene is lacking only intron/exon 4. Western blot analysis indicates that both *DHR96*^{E25} mutants, as well as *DHR96*^{16A} mutants, produce no detectable DHR96 protein. Thus, both alleles can be considered as null mutations.

78. One way to functionally test the mutants is in a viability assay based on different nutritional backgrounds. Disclosed herein, DHR96 mutants will have a decreased ability to grow on instant fly food, such as Carolina 424. If yeast is restored to the instant food, viability is restored to within wildtype levels, indicating that DHR96 mutants are sensitive to the absence of yeast in their food source. In contrast, mutants such as *DHR96*^{E25} or *DHR96*^{16A} are viable when grown on standard cornmeal medium.

79. Disclosed are insects, such as flies, containing the mutant DHR96 gene, as well as any of their developmental stages, such as larvae, eggs, or pupae. These flies can be used, for example, to be crossed with other strains of flies to make new strains harboring the DHR96 mutants. These strains could also be used, for example, as a type of insect inhibitor themselves, by being released in the wild to cross with wildtype insects creating mutant insects. For this purpose, mutations that create a dominant negative phenotype are preferred, such as those that have non-functional LBD, but retain their ability to heterodimerize, thus, interacting with and reducing the effect of native proteins in the insect.

80. The disclosed mutants cause a decrease in the insect's ability to react to toxins or pesticides, such as DDT. The disclosed mutants, such as *DHR96*^{16A} or *DHR96*^{E25} insects, such as flies, were more sensitive to DDT and died at lower concentrations of DDT compared to control animals (Fig. 4A). In addition, when challenged with a fixed concentration of DDT, *DHR96* homozygotes died more rapidly than wild type flies (Fig. 4B).

81. Also disclosed are mutants which have a defect in for example, activation with and without retention of dimerization ability, defects in ligand binding, and defects in DNA binding with and without loss of dimerization ability.

82. Also disclosed are mutants that, when overexpressed, fail to modulate genes in the xenobiotic pathway, such as genes in the four major detoxification families, cytochrome P450s,

carboxylesterases, glutathione S-transferases, and UDP-glucuronosyltransferases (Oakeshott JG, Home I, Sutherland TD, Russell RJ. The genomics of insecticide resistance. Genome Biol. 2003;4(1):202). In Table 3, two are P450s (Cyp genes), two are glutathione S-transferases , and one each of the carboxylesterases and UDP-glucuronosyltransferases were identified by microarray analysis. These represent the function of these proteins. Also denoted in Table 3 are the names of the genes. These are the gene names according to FlyBase (http://flybase.bio.indiana.edu/) They are either a proper name, like black or Lcp1, or the CG number, which is a numerical designation given to each fly gene. The CG number is usually used when the gene is new or of unknown function. This can be determined using microarrays as disclosed herein.

(4) Compounds that modulate DHR96 activity

83. Disclosed are compounds that modulate DHR96 activity. These compounds can, for example, modulate the activity of the protein through binding with the protein of DHR96, or through binding the mRNA of DHR96, and inhibiting the mRNA, through, for example, degradation or prevention of translation. The compositions can be any type of molecule, including, for example, proteins, small peptides, antibodies, functional nucleic acids, such as aptamers, antisense, ribozymes, dsRNA for RNAi or siRNA, or small molecules, such as those found in various combinatorial chemistry libraries or natural product libraries.

84. For example, disclosed are compounds that function by, for example, binding to the ligand binding domain of DHR96 and inactivating its function or turning it into a constitutive repressor, or mimicking the normal cofactors that mediate nuclear receptor signaling to the general transcription machinery. These compounds, such as peptides, would render the receptor incapable of directing proper target gene transcription, blocking the detoxification response. The disclosed compounds can act in combination with known or any pesticide by increasing the effectiveness of the pesticide by decreasing the insect's ability to react to the pesticide. The compositions could be added to pre-existing pesticide formulations, increasing their effectiveness. Moreover, resistant lines of insects that respond poorly to a particular pesticide may be made more sensitive by adding compounds that affect DHR96 function. DHR96 is a target for pest control, capable of regulating insect populations. The compositions could also prevent or reduce the translation or expression of the DHR96 mRNA, by for example, through RNAi or antisense mechanisms.

(a) Functional Nucleic Acids

85. Functional nucleic acids are nucleic acid molecules that have a specific function, such as binding a target molecule or catalyzing a specific reaction. Functional nucleic acid molecules can be divided into the following categories, which are not meant to be limiting. For example, functional nucleic acids include RNAi, antisense molecules, aptamers, ribozymes, triplex forming molecules, and external guide sequences. The functional nucleic acid molecules can act as affectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules can possess a de novo activity independent of any other molecules.

86. Functional nucleic acid molecules can interact with any macromolecule, such as DNA, RNA, polypeptides, or carbohydrate chains. Thus, functional nucleic acids can interact with the mRNA of DHR96 or variants or fragments or the genomic DNA of DHR96 or variants or fragments or they can interact with the polypeptide DHR96 or variants or fragments. Often functional nucleic acids are designed to interact with other nucleic acids based on sequence homology between the target molecule and the functional nucleic acid molecule. In other situations, the specific recognition between the functional nucleic acid molecule and the target molecule is not based on sequence homology between the functional nucleic acid molecule and the target molecule, but rather is based on the formation of tertiary structure that allows specific recognition to take place.

87. Disclosed are molecules that inhibit DHR96 activity that are based on RNA interference (RNAi) or small interfering RNA (SiRNA). It is thought that RNAi involves a two-step mechanism for RNA interference (RNAi): an initiation step and an effector step. For example, in the first step, input double-stranded (ds) RNA is processed into small fragments (siRNA), such as 21–23-nucleotide 'guide sequences'. RNA amplification appears to be able to occur in whole animals. Typically then, the guide RNAs can be incorporated into a protein RNA complex which is cable of degrading RNA, the nuclease complex, which has been called the RNA-induced silencing complex (RISC). This RISC complex acts in the second effector step to destroy mRNAs that are recognized by the guide RNAs through base-pairing interactions. RNAi involves the introduction by any means of double stranded RNA into the cell which triggers events that cause the degradation of a target RNA. RNAi is a form of post-transcriptional gene silencing. Disclosed are RNA hairpins that can act in RNAi.

88. RNAi has been shown to work in a number of cells, including mammalian and invertebrate cells. In certain embodiements the RNA molecules which will be used as targeting

sequences within the RISC complex are shorter. For example, less than or equal to 50 or 40 or 30 or 29, 28, 27, 26, 25, 24, 23, ,22, 21, 20, 19, 18, 17, 16 , 15, 14, 13 , 12, 11, or 10 nucleotides in length. These RNA molecules can also have overhangs on the 3' or 5' ends relative to the target RNA which is to be cleaved. These overhangs can be at least or less than or equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 nucleotides long.

89. Methods of RNAi and SiRNA are described in detail in Hannon et al. (2002), RNA Interference, Nature 418:244-250; Brummelkamp et al. (2002), A System for Stable Expression of Short Interfering RNAs in Mammalian Cells, Science 296:550-508; Paul et al. (2002), Effective expression of small interfering RNA in human cells, Nature Biotechnology 20: 505-508, which are each incorporated by reference in their entirety for methods of RNAi and SiRNA and for designing and testing various oligos useful therein.

90. RNA interference (RNAi) and gene targeting were used to disrupt *DHR96* function because no existing mutants were available. The effects of *DHR96* RNAi were analyzed by generating transgenic lines that express snapback RNA under the control of a heat-inducible promoter. Three independent lines showed strong reduction of *DHR96* mRNA in northern blots when treated with a single heat-shock, but displayed no discernable phenotype. Using a variety of heat-shock regimens, e.g. longer single and double treatments or 12 hr repetitions, did not affect the outcome of this observation. These findings suggest that *DHR96* mRNA is not necessary for viability under standard conditions, indicating either that DHR96 protein is very stable or dispensable for survival, and is consistent with the studies of *DHR96* null mutants.

91. Antisense molecules are designed to interact with a target nucleic acid molecule through either canonical or non-canonical base pairing. The interaction of the antisense molecule and the target molecule is designed to promote the destruction of the target molecule through, for example, RNAseH mediated RNA-DNA hybrid degradation. Alternatively the antisense molecule is designed to interrupt a processing function that normally would take place on the target molecule, such as transcription or replication. Antisense molecules can be designed based on the sequence of the target molecule. Numerous methods for optimization of antisense efficiency by finding the most accessible regions of the target molecule exist. Exemplary methods would be in vitro selection experiments and DNA modification studies using DMS and DEPC. It is preferred that antisense molecules bind the target molecule with a dissociation constant (k_d)less than or equal to 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. A representative sample of methods and techniques which aid in the design and use of antisense molecules can be found in the following non-limiting list of United States patents: 5,135,917, 5,294,533, 5,627,158, 5,641,754,

5,691,317, 5,780,607, 5,786,138, 5,849,903, 5,856,103, 5,919,772, 5,955,590, 5,990,088, 5,994,320, 5,998,602, 6,005,095, 6,007,995, 6,013,522, 6,017,898, 6,018,042, 6,025,198, 6,033,910, 6,040,296, 6,046,004, 6,046,319, and 6,057,437.

92. Aptamers are molecules that interact with a target molecule, preferably in a specific way. Typically aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets. Aptamers can bind small molecules, such as ATP (United States patent 5,631,146) and theophiline (United States patent 5,580,737), as well as large molecules, such as reverse transcriptase (United States patent 5,786,462) and thrombin (United States patent 5,543,293). Aptamers can bind very tightly with k_ds from the target molecule of less than 10^{-12} M. It is preferred that the aptamers bind the target molecule with a k_d less than 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. Aptamers can bind the target molecule with a very high degree of specificity. For example, aptamers have been isolated that have greater than a 10000 fold difference in binding affinities between the target molecule and another molecule that differ at only a single position on the molecule (United States patent 5,543,293). It is preferred that the aptamer have a k_d with the target molecule at least 10, 100, 1000, 10,000, or 100,000 fold lower than the k_d with a background binding molecule. It is preferred when doing the comparison for a polypeptide for example, that the background molecule be a different polypeptide. For example, when determining the specificity of aptamers to DHR96 protein or fragments or variants, the background protein could be serum albumin. Representative examples of how to make and use aptamers to bind a variety of different target molecules can be found in the following non-limiting list of United States patents: 5,476,766, 5,503,978, 5,631,146, 5,731,424 , 5,780,228, 5,792,613, 5,795,721, 5,846,713, 5,858,660 , 5,861,254, 5,864,026, 5,869,641, 5,958,691, 6,001,988, 6,011,020, 6,013,443, 6,020,130, 6,028,186, 6,030,776, and 6,051,698.

93. Ribozymes are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intramolecularly or intermolecularly. Ribozymes are thus catalytic nucleic acid. It is preferred that the ribozymes catalyze intermolecular reactions. There are a number of different types of ribozymes that catalyze nuclease or nucleic acid polymerase type reactions which are based on ribozymes found in natural systems, such as hammerhead ribozymes, (for example, but not limited to the following United States patents: 5,334,711, 5,436,330, 5,616,466, 5,633,133, 5,646,020, 5,652,094, 5,712,384, 5,770,715, 5,856,463, 5,861,288, 5,891,683, 5,891,684, 5,985,621, 5,989,908, 5,998,193, 5,998,203, WO 9858058 by Ludwig and Sproat, WO 9858057 by Ludwig and Sproat, and WO 9718312 by Ludwig and Sproat) hairpin

ribozymes (for example, but not limited to the following United States patents: 5,631,115, 5,646,031, 5,683,902, 5,712,384, 5,856,188, 5,866,701, 5,869,339, and 6,022,962), and tetrahymena ribozymes (for example, but not limited to the following United States patents: 5,595,873 and 5,652,107). There are also a number of ribozymes that are not found in natural systems, but which have been engineered to catalyze specific reactions de novo (for example, but not limited to the following United States patents: 5,580,967, 5,688,670, 5,807,718, and 5,910,408). Preferred ribozymes cleave RNA or DNA substrates, and more preferably cleave RNA substrates. Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. This recognition is often based mostly on canonical or non-canonical base pair interactions. This property makes ribozymes particularly good candidates for target specific cleavage of nucleic acids because recognition of the target substrate is based on the target substrates sequence. Representative examples of how to make and use ribozymes to catalyze a variety of different reactions can be found in the following non-limiting list of United States patents: 5,646,042, 5,693,535, 5,731,295, 5,811,300, 5,837,855, 5,869,253, 5,877,021, 5,877,022, 5,972,699, 5,972,704, 5,989,906, and 6,017,756.

94. Triplex forming functional nucleic acid molecules are molecules that can interact with either double-stranded or single-stranded nucleic acid. When triplex molecules interact with a target region, a structure called a triplex is formed, in which there are three strands of DNA forming a complex dependant on both Watson-Crick and Hoogsteen base-pairing. Triplex molecules are preferred because they can bind target regions with high affinity and specificity. It is preferred that the triplex forming molecules bind the target molecule with a k_d less than 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. Representative examples of how to make and use triplex forming molecules to bind a variety of different target molecules can be found in the following non-limiting list of United States patents: 5,176,996, 5,645,985, 5,650,316, 5,683,874, 5,693,773, 5,834,185, 5,869,246, 5,874,566, and 5,962,426.

95. External guide sequences (EGSs) are molecules that bind a target nucleic acid molecule forming a complex, and this complex is recognized by RNase P, which cleaves the target molecule. EGSs can be designed to specifically target a RNA molecule of choice. RNAse P aids in processing transfer RNA (tRNA) within a cell. Bacterial RNAse P can be recruited to cleave virtually any RNA sequence by using an EGS that causes the target RNA:EGS complex to mimic the natural tRNA substrate. (WO 92/03566 by Yale, and Forster and Altman, Science 238:407-409 (1990)).

96. Similarly, eukaryotic EGS/RNAse P-directed cleavage of RNA can be utilized to cleave desired targets within eukarotic cells. (Yuan et al., Proc. Natl. Acad. Sci. USA 89:8006-8010 (1992); WO 93/22434 by Yale; WO 95/24489 by Yale; Yuan and Altman, EMBO J 14:159-168 (1995), and Carrara et al., Proc. Natl. Acad. Sci. (USA) 92:2627-2631 (1995)). Representative examples of how to make and use EGS molecules to facilitate cleavage of a variety of different target molecules be found in the following non-limiting list of United States patents: 5,168,053, 5,624,824, 5,683,873, 5,728,521, 5,869,248, and 5,877,162.

(b) Antibodies

97. Disclosed are monoclonal and polyclonal as well as chimeric variants of these, that bind DHR96 or variants or fragments thereof. Also disclosed are monoclonal and polyclonal antibodies that bind DHR96 or variants or fragments thereof that inhibit DHR96 activity in, for example, the xenobiotic pathways disclosed herein. Various assays are disclosed herein that can be used to identify these antibodies, such as the nutritional viability assay disclosed herein or the sensitivity to toxins assay disclosed herein.

98. As used herein, the term "antibody" encompasses, but is not limited to, whole immunoglobulin (i.e., an intact antibody) of any class. Native antibodies are usually heterotetrameric glycoproteins, composed of two identical light (L) chains and two identical heavy (H) chains. Typically, each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V(H)) followed by a number of constant domains. Each light chain has a variable domain at one end (V(L)) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains. The light chains of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (k) and lambda (l), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of human immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG-1, IgG-2, IgG-3, and IgG-4; IgA-1 and IgA-2. One skilled in the art would recognize the comparable classes for mouse. The heavy chain constant domains that correspond to the

different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

99. The term "variable" is used herein to describe certain portions of the variable domains that differ in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not usually evenly distributed through the variable domains of antibodies. It is typically concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of the variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a b-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the b-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat E. A. et al., "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1987)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

100. As used herein, the term "antibody or fragments thereof" encompasses chimeric antibodies and hybrid antibodies, with dual or multiple antigen or epitope specificities, and fragments, such as F(ab')2, Fab', Fab and the like, including hybrid fragments. Thus, fragments of the antibodies that retain the ability to bind their specific antigens are provided. For example, fragments of antibodies which maintain binding activity to the DHR96 or variants or fragments thereof are included within the meaning of the term "antibody or fragment thereof." Such antibodies and fragments can be made by techniques known in the art and can be screened for specificity and activity according to the methods set forth in the Examples and in general methods for producing antibodies and screening antibodies for specificity and activity (See Harlow and Lane. Antibodies, A Laboratory Manual. Cold Spring Harbor Publications, New York, (1988)).

101. Also included within the meaning of "antibody or fragments thereof" are conjugates of antibody fragments and antigen binding proteins (single chain antibodies) as described, for example, in U.S. Pat. No. 4,704,692, the contents of which are hereby incorporated by reference.

102. Optionally, the antibodies are generated in other species and "humanized" for administration in humans. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

103. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

104. The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important in order to reduce antigenicity. According to

the "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol., 151:2296 (1993) and Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol., 151:2623 (1993)).

105. It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (see, WO 94/04679, published 3 March 1994).

106. Transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be employed. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J(H)) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551-255 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggemann et al., Year in Immuno., 7:33 (1993)). Human antibodies can also be produced in phage display libraries (Hoogenboom et al., J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). The techniques of Cote et al. and Boerner et al. are also available for the

preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(1):86-95 (1991)).

107. Disclosed are hybidoma cells that produces the monoclonal antibody. The term "monoclonal antibody" as used herein refers to an antibody obtained from a substantially homogeneous population of antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired activity (See, U.S. Pat. No. 4,816,567 and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

108. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975) or Harlow and Lane. Antibodies, A Laboratory Manual. Cold Spring Harbor Publications, New York, (1988). In a hybridoma method, a mouse or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized in vitro. Preferably, the immunizing agent comprises DHR96 or variants or fragments thereof. Traditionally, the generation of monoclonal antibodies has depended on the availability of purified protein or peptides for use as the immunogen. More recently DNA based immunizations have shown promise as a way to elicit strong immune responses and generate monoclonal antibodies. In this approach, DNA-based immunization can be used, wherein DNA encoding a portion of DHR96 or variants or fragments thereof expressed as a fusion protein with human IgG1 is injected into the host animal according to methods known in the art (e.g., Kilpatrick KE, et al. Gene gun delivered DNA-based immunizations mediate rapid production of murine monoclonal antibodies to the Flt-3 receptor. Hybridoma. 1998 Dec;17(6):569-76; Kilpatrick KE et al. High-affinity monoclonal antibodies to PED/PEA-15 generated using 5 microg of DNA. Hybridoma. 2000 Aug;19(4):297-302, which are incorporated herein by referenced in full for the the methods of antibody production) and as described in the examples.

109. An alternate approach to immunizations with either purified protein or DNA is to use antigen expressed in baculovirus. The advantages to this system include ease of generation, high levels of expression, and post-translational modifications that are highly similar to those seen in mammalian systems. Use of this system involves expressing domains of antibodies to DHR96 or variants or fragments thereof as fusion proteins. The antigen is produced by inserting a gene fragment in-frame between the signal sequence and the mature protein domain of the antibodies to DHR96 or variants or fragments thereof nucleotide sequence. This results in the display of the foreign proteins on the surface of the virion. This method allows immunization with whole virus, eliminating the need for purification of target antigens.

110. Generally, either peripheral blood lymphocytes ("PBLs") are used in methods of producing monoclonal antibodies if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, "Monoclonal Antibodies: Principles and Practice" Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, including myeloma cells of rodent, bovine, equine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells. Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Rockville, Md. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., "Monoclonal Antibody Production Techniques and Applications" Marcel Dekker, Inc., New York, (1987) pp. 51-63). The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against DHR96 or variants or fragments thereof. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation

or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art, and are described further in the Examples below or in Harlow and Lane "Antibodies, A Laboratory Manual" Cold Spring Harbor Publications, New York, (1988).

111. After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution or FACS sorting procedures and grown by standard methods. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown in vivo as ascites in a mammal.

112. The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, protein G, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

113. The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567. DNA encoding the monoclonal antibodies can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, plasmacytoma cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Optionally, such a non-immunoglobulin polypeptide is substituted for the constant domains of an antibody or substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for DHR96 or variants or fragments thereof and another antigen-combining site having specificity for a different antigen.

114. *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Examples of papain digestion are described in WO 94/29348 published Dec. 22, 1994,

U.S. Pat. No. 4,342,566, and Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, (1988). Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields a fragment, called the F(ab')2 fragment, that has two antigen combining sites and is still capable of cross-linking antigen.

115. The Fab fragments produced in the antibody digestion also contain the constant domains of the light chain and the first constant domain of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain domain including one or more cysteines from the antibody hinge region. The F(ab')2 fragment is a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

116. An isolated immunogenically specific paratope or fragment of the antibody is also provided. A specific immunogenic epitope of the antibody can be isolated from the whole antibody by chemical or mechanical disruption of the molecule. The purified fragments thus obtained are tested to determine their immunogenicity and specificity by the methods taught herein. Immunoreactive paratopes of the antibody, optionally, are synthesized directly. An immunoreactive fragment is defined as an amino acid sequence of at least about two to five consecutive amino acids derived from the antibody amino acid sequence.

117. One method of producing proteins comprising the antibodies is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the antibody, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of an antibody can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H.

Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY. Alternatively, the peptide or polypeptide is independently synthesized in vivo as described above. Once isolated, these independent peptides or polypeptides may be linked to form an antibody or fragment thereof via similar peptide condensation reactions.

118. For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two step chemical reaction (Dawson et al. Synthesis of Proteins by Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide-alpha-thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site. Application of this native chemical ligation method to the total synthesis of a protein molecule is illustrated by the preparation of human interleukin 8 (IL-8) (Baggiolini M et al. (1992) FEBS Lett. 307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al., Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

119. Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

120. Also disclosed are fragments of antibodies which have bioactivity. The polypeptide fragments can be recombinant proteins obtained by cloning nucleic acids encoding the polypeptide in an expression system capable of producing the polypeptide fragments thereof, such as an adenovirus or baculovirus expression system. For example, one can determine the active domain of an antibody from a specific hybridoma that can cause a biological effect associated with the interaction of the antibody with DHR96 or variants or fragments thereof. For example, amino acids found to not contribute to either the activity or the binding specificity or

affinity of the antibody can be deleted without a loss in the respective activity. For example, in various embodiments, amino or carboxy-terminal amino acids are sequentially removed from either the native or the modified non-immunoglobulin molecule or the immunoglobulin molecule and the respective activity assayed in one of many available assays. In another example, a fragment of an antibody comprises a modified antibody wherein at least one amino acid has been substituted for the naturally occurring amino acid at a specific position, and a portion of either amino terminal or carboxy terminal amino acids, or even an internal region of the antibody, has been replaced with a polypeptide fragment or other moiety, such as biotin, which can facilitate in the purification of the modified antibody. For example, a modified antibody can be fused to a maltose binding protein, through either peptide chemistry or cloning the respective nucleic acids encoding the two polypeptide fragments into an expression vector such that the expression of the coding region results in a hybrid polypeptide. The hybrid polypeptide can be affinity purified by passing it over an amylose affinity column, and the modified antibody receptor can then be separated from the maltose binding region by cleaving the hybrid polypeptide with the specific protease factor Xa. (See, for example, New England Biolabs Product Catalog, 1996, pg. 164.). Similar purification procedures are available for isolating hybrid proteins from eukaryotic cells as well.

121. The fragments, whether attached to other sequences or not, include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the fragment is not significantly altered or impaired compared to the nonmodified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove or add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the fragment must possess a bioactive property, such as binding activity, regulation of binding at the binding domain, etc. Functional or active regions of the antibody may be identified by mutagenesis of a specific region of the protein, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the antigen. (Zoller MJ et al. Nucl. Acids Res. 10:6487-500 (1982).

122. A variety of immunoassay formats may be used to select antibodies that selectively bind with a particular protein, variant, or fragment. For example, solid-phase ELISA immunoassays are routinely used to select antibodies selectively immunoreactive with a protein, protein variant, or fragment thereof. See Harlow and Lane. Antibodies, A Laboratory Manual.

Cold Spring Harbor Publications, New York, (1988), for a description of immunoassay formats and conditions that could be used to determine selective binding. The binding affinity of a monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., Anal. Biochem., 107:220 (1980).

123. Also provided is an antibody reagent kit comprising containers of the monoclonal antibody or fragment thereof and one or more reagents for detecting binding of the antibody or fragment thereof to DHR96 or variants or fragments thereof. The reagents can include, for example, fluorescent tags, enzymatic tags, or other tags. The reagents can also include secondary or tertiary antibodies or reagents for enzymatic reactions, wherein the enzymatic reactions produce a product that can be visualized.

(c) Compositions identified by screening with disclosed compositions / combinatorial chemistry

(i) Combinatorial chemistry

124. The disclosed compositions can be used as targets for any combinatorial technique to identify molecules or macromolecular molecules that interact with the disclosed compositions in a desired way. The nucleic acids, peptides, and related molecules disclosed herein, such as DHR96 or variants or fragments thereof, can be used as targets for the combinatorial approaches. Also disclosed are the compositions that are identified through combinatorial techniques or screening techniques in which the compositions, such as DHR96 or variants or fragments thereof, or portions thereof, are used as the target in a combinatorial or screening protocol.

125. It is understood that when using the disclosed compositions in combinatorial techniques or screening methods, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition or stimulation or the target molecule's function. The molecules identified and isolated when using the disclosed compositions, such as, DHR96 or variants or fragments thereof, are also disclosed. Thus, the products produced using the combinatorial or screening approaches that involve the disclosed compositions, such as, DHR96 or variants or fragments thereof, are also considered herein disclosed.

126. It is understood that the disclosed methods for identifying molecules that inhibit the interactions between, for example, DHR96 or variants or fragments thereof, can be performed using high through put means. For example, putative inhibitors can be identified using Fluorescence Resonance Energy Transfer (FRET) to quickly identify interactions. The

underlying theory of the techniques is that when two molecules are close in space, ie, interacting at a level beyond background, a signal is produced or a signal can be quenched. Then, a variety of experiments can be performed, including, for example, adding in a putative inhibitor. If the inhibitor competes with the interaction between the two signaling molecules, the signals will be removed from each other in space, and this will cause a decrease or an increase in the signal, depending on the type of signal used. This decrease or increasing signal can be correlated to the presence or absence of the putative inhibitor. Any signaling means can be used. For example, disclosed are methods of identifying an inhibitor of the interaction between any two of the disclosed molecules comprising, contacting a first molecule and a second molecule together in the presence of a putative inhibitor, wherein the first molecule or second molecule comprises a fluorescence donor, wherein the first or second molecule, typically the molecule not comprising the donor, comprises a fluorescence acceptor; and measuring Fluorescence Resonance Energy Transfer (FRET), in the presence of the putative inhibitor and the in absence of the putative inhibitor, wherein a decrease in FRET in the presence of the putative inhibitor as compared to FRET measurement in its absence indicates the putative inhibitor inhibits binding between the two molecules. This type of method can be performed with a cell system as well.

127. Combinatorial chemistry includes but is not limited to all methods for isolating small molecules or macromolecules that are capable of binding either a small molecule or another macromolecule, typically in an iterative process. Proteins, oligonucleotides, and sugars are examples of macromolecules. For example, oligonucleotide molecules with a given function, catalytic or ligand-binding, can be isolated from a complex mixture of random oligonucleotides in what has been referred to as "in vitro genetics" (Szostak, TIBS 19:89, 1992). One synthesizes a large pool of molecules bearing random and defined sequences and subjects that complex mixture, for example, approximately 10^{15} individual sequences in 100 μg of a 100 nucleotide RNA, to some selection and enrichment process. Through repeated cycles of affinity chromatography and PCR amplification of the molecules bound to the ligand on the column, Ellington and Szostak (1990) estimated that 1 in 10^{10} RNA molecules folded in such a way as to bind a small molecule dyes. DNA molecules with such ligand-binding behavior have been isolated as well (Ellington and Szostak, 1992; Bock et al, 1992). Techniques aimed at similar goals exist for small organic molecules, proteins, antibodies and other macromolecules known to those of skill in the art. Screening sets of molecules for a desired activity whether based on small organic libraries, oligonucleotides, or antibodies is broadly referred to as combinatorial chemistry. Combinatorial techniques are particularly suited for defining binding interactions

between molecules and for isolating molecules that have a specific binding activity, often called aptamers when the macromolecules are nucleic acids.

128. There are a number of methods for isolating proteins which either have de novo activity or a modified activity. For example, phage display libraries have been used to isolate numerous peptides that interact with a specific target. (See for example, United States Patent No. 6,031,071; 5,824,520; 5,596,079; and 5,565,332 which are herein incorporated by reference at least for their material related to phage display and methods relate to combinatorial chemistry)

129. A preferred method for isolating proteins that have a given function is described by Roberts and Szostak (Roberts R.W. and Szostak J.W. Proc. Natl. Acad. Sci. USA, 94(23)12997-302 (1997). This combinatorial chemistry method couples the functional power of proteins and the genetic power of nucleic acids. An RNA molecule is generated in which a puromycin molecule is covalently attached to the 3'-end of the RNA molecule. An *in vitro* translation of this modified RNA molecule causes the correct protein, encoded by the RNA to be translated. In addition, because of the attachment of the puromycin, a peptdyl acceptor which cannot be extended, the growing peptide chain is attached to the puromycin which is attached to the RNA. Thus, the protein molecule is attached to the genetic material that encodes it. Normal *in vitro* selection procedures can now be done to isolate functional peptides. Once the selection procedure for peptide function is complete traditional nucleic acid manipulation procedures are performed to amplify the nucleic acid that codes for the selected functional peptides. After amplification of the genetic material, new RNA is transcribed with puromycin at the 3'-end, new peptide is translated and another functional round of selection is performed. Thus, protein selection can be performed in an iterative manner just like nucleic acid selection techniques. The peptide which is translated is controlled by the sequence of the RNA attached to the puromycin. This sequence can be anything from a random sequence engineered for optimum translation (i.e. no stop codons etc.) or it can be a degenerate sequence of a known RNA molecule to look for improved or altered function of a known peptide. The conditions for nucleic acid amplification and in vitro translation are well known to those of ordinary skill in the art and are preferably performed as in Roberts and Szostak (Roberts R.W. and Szostak J.W. Proc. Natl. Acad. Sci. USA, 94(23)12997-302 (1997)).

130. Another preferred method for combinatorial methods designed to isolate peptides is described in Cohen et al. (Cohen B.A.,et al., Proc. Natl. Acad. Sci. USA 95(24):14272-7 (1998)). This method utilizes and modifies two-hybrid technology. Yeast two-hybrid systems are useful for the detection and analysis of protein:protein interactions. The two-hybrid system,

initially described in the yeast *Saccharomyces cerevisiae,* is a powerful molecular genetic technique for identifying new regulatory molecules, specific to the protein of interest (Fields and Song, *Nature* 340:245-6 (1989)). Cohen et al., modified this technology so that novel interactions between synthetic or engineered peptide sequences could be identified which bind a molecule of choice. The benefit of this type of technology is that the selection is done in an intracellular environment. The method utilizes a library of peptide molecules that attached to an acidic activation domain. A peptide of choice, for example, of DHR96 or variants or fragments thereof, is attached to a DNA binding domain of a transcriptional activation protein, such as Gal 4. By performing the two-hybrid technique on this type of system, molecules that bind DHR96 or variants or fragments thereof can be identified.

131. Using methodology well known to those of skill in the art, in combination with various combinatorial libraries, one can isolate and characterize those small molecules or macromolecules, which bind to or interact with the desired target. The relative binding affinity of these compounds can be compared and optimum compounds identified using competitive binding studies, which are well known to those of skill in the art.

132. Techniques for making combinatorial libraries and screening combinatorial libraries to isolate molecules which bind a desired target are well known to those of skill in the art. Representative techniques and methods can be found in but are not limited to United States patents 5,084,824, 5,288,514, 5,449,754, 5,506,337, 5,539,083, 5,545,568, 5,556,762, 5,565,324, 5,565,332, 5,573,905, 5,618,825, 5,619,680, 5,627,210, 5,646,285, 5,663,046, 5,670,326, 5,677,195, 5,683,899, 5,688,696, 5,688,997, 5,698,685, 5,712,146, 5,721,099, 5,723,598, 5,741,713, 5,792,431, 5,807,683, 5,807,754, 5,821,130, 5,831,014, 5,834,195, 5,834,318, 5,834,588, 5,840,500, 5,847,150, 5,856,107, 5,856,496, 5,859,190, 5,864,010, 5,874,443, 5,877,214, 5,880,972, 5,886,126, 5,886,127, 5,891,737, 5,916,899, 5,919,955, 5,925,527, 5,939,268, 5,942,387, 5,945,070, 5,948,696, 5,958,702, 5,958,792, 5,962,337, 5,965,719, 5,972,719, 5,976,894, 5,980,704, 5,985,356, 5,999,086, 6,001,579, 6,004,617, 6,008,321, 6,017,768, 6,025,371, 6,030,917, 6,040,193, 6,045,671, 6,045,755, 6,060,596, and 6,061,636.

133. Combinatorial libraries can be made from a wide array of molecules using a number of different synthetic techniques. For example, libraries containing fused 2,4-pyrimidinediones (United States patent 6,025,371) dihydrobenzopyrans (United States Patent 6,017,768and 5,821,130), amide alcohols (United States Patent 5,976,894), hydroxy-amino acid amides (United States Patent 5,972,719) carbohydrates (United States patent 5,965,719), 1,4-benzodiazepin-2,5-diones (United States patent 5,962,337), cyclics (United States patent

5,958,792), biaryl amino acid amides (United States patent 5,948,696), thiophenes (United States patent 5,942,387), tricyclic Tetrahydroquinolines (United States patent 5,925,527), benzofurans (United States patent 5,919,955), isoquinolines (United States patent 5,916,899), hydantoin and thiohydantoin (United States patent 5,859,190), indoles (United States patent 5,856,496), imidazol-pyrido-indole and imidazol-pyrido-benzothiophenes (United States patent 5,856,107) substituted 2-methylene-2, 3-dihydrothiazoles (United States patent 5,847,150), quinolines (United States patent 5,840,500), PNA (United States patent 5,831,014), containing tags (United States patent 5,721,099), polyketides (United States patent 5,712,146), morpholino-subunits (United States patent 5,698,685 and 5,506,337), sulfamides (United States patent 5,618,825), and benzodiazepines (United States patent 5,288,514).

134. As used herein combinatorial methods and libraries included traditional screening methods and libraries as well as methods and libraries used in interative processes.

(ii) Computer assisted drug design

135. The disclosed compositions can be used as targets for any molecular modeling technique to identify either the structure of the disclosed compositions or to identify potential or actual molecules, such as small molecules, which interact in a desired way with the disclosed compositions. The nucleic acids, peptides, and related molecules disclosed herein, such as DHR96 or variants or fragments thereof, can be used as targets in any molecular modeling program or approach.

136. It is understood that when using the disclosed compositions in modeling techniques, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition or stimulation or the target molecule's function. The molecules identified and isolated when using the disclosed compositions, such as, DHR96 or variants or fragments thereof, are also disclosed. Thus, the products produced using the molecular modeling approaches that involve the disclosed compositions, such as, DHR96 or variants or fragments thereof, are also considered herein disclosed.

137. Thus, one way to isolate molecules that bind a molecule of choice is through rational design. This is achieved through structural information and computer modeling. Computer modeling technology allows visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds that will interact with the molecule. The three-dimensional construct typically depends on data from x-ray crystallographic analyses or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new compound will link to the

target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity. Prediction of what the molecule-compound interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

138. Examples of molecular modeling systems are the CHARMm and QUANTA programs, Polygen Corporation, Waltham, MA. CHARMm performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

139. A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen, et al., 1988 *Acta Pharmaceutica Fennica* 97, 159-166; Ripka, *New Scientist* 54-57 (June 16, 1988); McKinaly and Rossmann, 1989 *Annu. Rev. Pharmacol. Toxiciol.* 29, 111-122; Perry and Davies, <u>QSAR: Quantitative Structure-Activity Relationships in Drug Design</u> pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 *Proc. R. Soc. Lond.* 236, 125-140 and 141-162; and, with respect to a model enzyme for nucleic acid components, Askew, et al., 1989 *J. Am. Chem. Soc.* 111, 1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, CA., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario. Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of molecules specifically interacting with specific regions of DNA or RNA, once that region is identified.

140. Although described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which alter substrate binding or enzymatic activity.

(5) Insects that can be targeted

141. Arthropods include Crustacea, which are things like prawns, crabs and woodlice; Myriapoda, which are centipedes, millipedes and such; Chelicerata (Arachnida), which are spiders, scorpions and harvestmen etc., and Uniramia (Insecta), which are things like beetles, bees and flies.

142. Insects are found in the phylum Arthorpoda, Subphylum Insecta (also often called a class), Class Hexapoda, and Subclasses Apterygota, Exopterygota, and Endopterygota. The

Apterygota includes the orders Protura, Collembola (Springtails), Thysanura (Silverfish), Diplura (Two Pronged Bristle-tails). The Exopterygota includes the orders Ephemeroptera (Mayflies), Odonata (Dragonflies), Plecoptera (Stoneflies), Grylloblatodea, Orthoptera, Phasmida (Stick-Insects), Dermaptera (Earwigs), Embioptera (Web Spinners), Dictyoptera (Cockroaches and Mantids), Isoptera (Termites), Zoraptera, Psocoptera (Bark and Book Lice), Mallophaga (Biting Lice), Siphunculata (Sucking Lice), Hemiptera (True Bugs) Thysanoptera, The Endopterygota includes the orders Neuropter (Lacewings), Coleoptera (Beetles), Strepsiptera (Stylops), Mecoptera (Scorpionflies), Siphonaptera (Fleas), Diptera (True Flies which are unusual in that they only have one pair of functional wings. The other pair is reduced to a pair of knoblike organs, called halteres, which play a part in stabilizing these insects during flight. True flies include house flies and bluebottles, mosquitoes, horseflies, midges, and antler-headed flies), Lepidoptera (Butterflies and Moths), Trichoptera (Caddis Flies), and Hymenoptera (Ants Bees and Wasps).

(6) Exemplary pesticides that can be used in combination

143. The disclosed compositions, such as DHR96 inhibitors can be combined with any pesticide or class of pesticides. For example, the DHR96 inhibitors can be combined with a pesticide that invokes the xenobiotic pathway. The DHR96 inhibitors can also be combined with any pesticide that effects the expression of a gene in the following four familes, cytochrome P450s, carboxylesterases, glutathione S-transferases, and UDP-glucuronosyltransferases When it is unknown which xenobiotic genes are affected by the pesticide, this can be determined by observing whether the pesticide turns on one or more genes that are in the xenobiotic pathway, by for example, microarray technology, or any other technology that determines gene expression, such as RT-PCR. In certain embodiements, when a particular gene product is specifically overexpressed in a resistant line of insects, that gene product can be considered a xenobiotic gene. Other examples, such as cuticle proteins and a serum carrier protein, were seen in the microarray experiments as well. In other embodiements any encoded protein that confers resistance to a toxic compound can be considered a xenobiotic compound.

144. There are many different pesticides that are relatively common chemicals, such as arsenicals, petroleum oils, nicotine, pyrethrum, rotenone, sulfur, hydrogen cyanide gas, and cryolite. However, most pesticides are non-natural chemically synthesized compounds. For example, there are different classes and subclasses of pesticides, such as organochlorines, examples of which are diphenyl aliphatics, hexchlorocyclohexane (HCH) or benzenehexachloride (BHC), Cyclodienes, Polychloroterpenes, organophosphates (OPs)

examples of whch are esters of phosphorus, organosulfers, carbamates, formamidines, dinitrophenols, oganotins, pyrethroids, nicotinoids (also known as nitro-quanidines, neonicotinyls, neonicotinoids, chloronicotines, or chloronicotinyls), spinosyns, fiproles (or Phenylpyrazoles), pyrroles, pyrazoles, pyridazinones, quinazolines, benzoylureas, botanicals, (natural insecticides), synergists or activators, antibiotics, fumigants, insect repellants, and inorganics.

145. Another way of classifying insecticides is by their mode of action, for example, sodium and/or potassium channel inhibitors, buerotoxins, GABA (gamma-aminobutyric acid) receptor modulators, such as inhibitors and activators, cholinesterase (ChE) inhibitors, aliesterase inhibitors, monoamine oxidase inhibitors, oxidative phosphorylation couplers or uncouplers, adenosine triphosphate (ATP) formation inhibitors, dinitrophenol uncoupling inhibitors, axionic poisons, inhibition of postsynaptic nicotinergic acetylcholine receptors, inhibiting of binding of acetylcholine in nicotinic acetylcholine receptors at the postsynaptic cell, inhibition of gamma-aminobutyric acid- (GABA) regulated chloride channels in neurons, inhibitors of mitochondrial electron transport at the NADH-CoQ reductase site, general inhibitors of mitochondrial electron transport at Site 1, insect growth regulators (IGR, inhibitors of various life cycles and stages in the insect), chitin synthesis inhibitors, inhibitors of exoskeleton development, respiratory enzyme inhibitors, inhibitors of the interaction between NAD+ and coenzyme Q, .inhibitors of molting, inhibitors of the biosynthesis or metabolism of ecdysone, synergists, such as inhibitors of cytochrome P-450 dependent polysubstrate monooxygenases (PSMOs), and narcotics, calcium channel inhibitors, and repellants.

146. Examples of organochlorines are (chlorinated hydrocarbons, chlorinated organics, chlorinated insecticides, and chlorinated synthetics) Diphenyl Aliphatics, such as DDT, DDD, dicofol, ethylan, chlorobenzilate, and methoxychlor, Hexchlorocyclohexanes (HCH) or benzenehexachloride (BHC), which are typically gamma isomers, such as lindane, Cyclodienes, such as chlordane, aldrin and dieldrin, heptachlor, endrin, mirex, endosulfan, and chlordecone (Kepone®), and Polychloroterpenes, such as toxaphene and strobane.

147. Examples of organophosphates (OPs) examples of which are esters of phosphorus, (also called organic phosphates, phosphorus insecticides, nerve gas relatives, and phosphoric acid esters) derived from phosphorus acids, such as sarin, soman, and tabun, subclasses included phosphates, phospho-nates, phosphorothioates, phosphorodithioates, phosphorothiolates and phosphoramidates. There are also aliphatic, phenyl, and heterocyclic derivatives. The aliphatics include TEPP, malathion, trichlorfon (Dylox®), monocrotophos

(Azodrin®), dimethoate (Cygon®), oxydemetonmethyl (Meta Systox®), dimethoate (Cygon®), dicrotophos (Bidrin®), disulfoton (Di-Syston®), dichlorvos (Vapona®), mevinphos (Phosdrin®), methamidophos (Monitor®), and acephate (Orthene®). The Phenyl derivatives parathion (ethyl parathion), methyl parathion, profenofos (Curacron®), sulprofos (Bolstar®), isofenphos (Oftanol®, Pryfon®), fenitrothion (Sumithion®), fenthion (Dasanit®), famphur (Cyflee® and Warbex®). The Heterocyclic derivatives include diazinon, azinphos-methyl (Guthion®), azinphos-ethyl (Acifon®, Gusathion®), chlorpyrifos (Dursban®, Lorsban®, Lock-On®), methidathion (Supracide®), phosmet (Imidan®), isazophos (Brace®, Triumph®), and chlorpyrifos-methyl (Reldan®).

148. Examples of organosulfers typically contain two phenyl rings, resembling DDT, with sulfur in place of carbon as the central atom, and include tetradifon (Tedion®), propargite (Omite®, Comite®), and ovex (Ovotran®).

149. Examples of carbamates are derivatives of carbamic acid and include carbaryl (Sevin®), methomyl (Lannate®), carbofuran (Furadan®), aldicarb (Temik®), oxamyl (Vydate®), thiodicarb (Larvin®), methiocarb (Mesurol®), propoxur (Baygon®), bendiocarb (Ficam®), carbosulfan (Advantage®), aldoxycarb (Standak®), promecarb (Carbamult®), and fenoxycarb (Logic®, Torus®).

150. Examples of formamidines include chlordimeform (Galecron®, Fundal®), formetanate (Carzol®), and amitraz (Mitac®, Ovasyn®.

151. Examples of dinitrophenols include binapacryl (Morocide®) and dinocap (Karathane®).

152. Examples of oganotins include cyhexatin (Plictran®) and Fenbutatin-oxide (Vendex®).

153. Examples of pyrethroids natural pyrethrum and synthetic pyrethroids including allethrin (Pynamin®), tetramethrin (Neo-Pynamin®) (1965), resmethrin (Synthrin®), bioresmethrin, Bioallethrin®, phonothrin (Sumithrin®), fenvalerate (Pydrin®, Tribute®, & Bellmark®), permethrin (Ambush®, Astro®, Dragnet®, Flee®, Pounce®, Prelude®, Talcord® & Torpedo®), bifenthrin (Capture®, Talstar®), *lambda*-cyhalothrin (Demand®, Karate®, Scimitar® & Warrior®), cypermethrin (Ammo®, Barricade®,Cymbush®, Cynoff® & Ripcord®), cyfluthrin (Baythroid®, Countdown®, Cylense®, Laser® & Tempo®), deltamethrin (Decis®) esfenvalerate (Asana®, Hallmark®), fenpropathrin (Danitol®), flucythrinate (Cybolt®, Payoff®), fluvalinate (Mavrik®, Spur ®), prallethrin (Etoc®), *tau*-fluvalinate (Mavrik®)

tefluthrin (Evict®, Fireban®, Force® & Raze®), tralomethrin (Scout X-TRA®, Tralex®), and *zeta*-cypermethrin (Mustang® Fury®), acrinathrin (Rufast®), and imiprothrin (Pralle®.

154. Examples of nicotinoids (also known as nitro-quanidines, neonicotinyls, neonicotinoids, chloronicotines, or chloronicotinyls) including Imidacloprid (Admire®, Confidor®,Gaucho®, Merit®, Premier®, Premise® and Provado®), acetamiprid (Mospilan®), thiamethoxam (Actara®, Platinum®), and nitenpyram (Bestguard®).

155. Examples of spinosyns include (Success®, Tracer Naturalyte®).

156. Examples of fiproles (or Phenylpyrazoles) include Fipronil ((Regent®, Icon®, Frontline®).

157. Examples of pyrroles include Chlorfenapyr ((Alert®, Pirate®.

158. Examples of pyrazoles include tebufenpyrad (Pyranica®, Masai®) and fenpyroximate (Acaban®, Dynamite®).

159. Examples of pyridazinones include Pyridaben ((Nexter®, Sanmite®).

160. Examples of quinazolines fenazaquin ((Matador®).

161. Examples of benzoylureas include triflumuron (Alsystin®), chlorfluazuron (Atabron®, Helix®), followed by teflubenzuron (Nomolt®, Dart®), hexaflumuron (Trueno®, Consult®), flufenoxuron (Cascade®), flucycloxuron (Andalin®), flurazuron, novaluron, diafenthiuron, Lufenuron (Axor®), and diflubenzuron ((Dimilin®, Adept®, Micromite®).

162. Examples of botanicals, (natural insecticides) include sulfur, tobacco, pyrethrum, derris, hellebore, quassia, camphor, and turpentine, and Pyrethrum, alkaloids, such as nicotine, caffeine (coffee, tea), quinine (cinchona bark), morphine (opium poppy), cocaine (coca leaves), ricinine (a poison in castor oil beans), strychnine (*Strychnos nux vomica*), coniine (spotted hemlock, the poison used by Socrates), and LSD (a hallucigen from the ergot fungus attacking grain), rotenone, Limonene or d-Limonene, neem, Azadirachtin (Azatin® is marketed as an insect growth regulator, and Align® and Nemix®).

163. Examples of synergists or activators are not insecticides per se, but rather enhance the activity of insecticides having a primary insecticidal effect. Examples include, piperonyl butoxide, and contain the methylenedioxyphenyl moiety (found in sesame seed oil (*sesamin)).*

164. Examples of antibiotics include avermectins, Abamectin, Clinch®, Emamectin benzoate (Proclaim®, Denim®).

165. Examples of fumigants typically contain one or more halogens, such as methyl bromide (Aspelin and Grube 1998), ethylene dichloride, hydrogen cyanide, sulfuryl fluoride (Vikane®), Vapam®, Telone® II, D-D®, chlorothene, ethylene oxide, napthalene crystals,

paradichlorobenzene crystals, Phosphine gas (PH_3) produced by alunimum or magnesium phosphide pellets.

166. Examples of insect repellants include dimethyl phthalate, Indalone®, Rutgers 612®, dibutyl phthalate, various MGK® repellents, benzyl benzoate, the military clothing repellent (N-butyl acetanilide), dimethyl carbate (Dimelone®) and diethyl toluamide (DEET, Delphene®).

167. Examples of inorganics include sulfur, mercury, boron, thallium, arsenic, antimony, selenium, and fluoride, arsenicals, including copper arsenate, Paris green, lead arsenate, and calcium arsenate, inorganic fluorides such as sodium fluoride, barium fluosilicate, sodium silicofluoride, and cryolite (Kryocide®), Boric acid, Sodium borate (disodium octaborate tetrahydrate) (Tim-Bor®, Bora-Care®), silica gels or silica aerogels, such as Dri-Die®, Drianone®, and Silikil Microcel®.

168. Other compounds not easily categorized include cyromazine (Larvadex®, Trigard®), a triazine, pyriproxyfen (Knack®, Esteem®, Archer®), insect growth inhibitors such as buprofezin (Applaud®) and thiadiazines, tetrazines, such as clofentezine (Apollo®, Acaristop®), Enzone®, sodium tetrathiocarbonate, and Clandosan®.

169. Also used are Veratrum Alkaloids, such as sabadilla, veratridine, and cevadine.

170. Also used are ryanoids, such as ryanodine, 10-(*O*-methyl)-ryanodine, 9,21-dehydroryanodine, ryanodol, and 9,21-dehydroryanodine.

171. Also used are octopamines mimics, such as amitraz® and chlordimeform.

172. Also included are respiration inhibitors, such as fenazaquin, pyridaben, amidinohydrazone, hydramethylnon and the perfluorooctanesulfonamide, and sulfluramid.

173. Also included are juvenile hormone mimics, such a juvenile hormone III, methoprene, and fenoxycarb.

174. Also included are toxins produced by Bacillus thuringiensis, such as Dipel®, Javelin®, Agree®.

C. Compositions

175. Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a

particular DHR96 or variants or fragments thereof is disclosed and discussed and a number of modifications that can be made to a number of molecules including the DHR96 or variants or fragments thereof are discussed, specifically contemplated is each and every combination and permutation of DHR96 or variants or fragments thereof and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

1. Sequence similarities

176. It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

177. In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. In general, variants of genes and proteins herein disclosed typically have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence or the native sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

178. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

179. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

180. For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

2. Hybridization/selective hybridization

181. The term hybridization typically means a sequence driven interaction between at least two nucleic acid molecules, such as a primer or a probe and a gene. Sequence driven interaction means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide derivatives in a nucleotide specific manner. For example, G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two nucleic acid molecules will hybridize.

182. Parameters for selective hybridization between two nucleic acid molecules are well known to those of skill in the art. For example, in some embodiments selective hybridization conditions can be defined as stringent hybridization conditions. For example, stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. For example, the conditions of hybridization to achieve selective hybridization may involve hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the Tm (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C below the Tm. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The conditions can be used as described above to achieve stringency, or as is known in the art. (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. Methods Enzymol. 1987:154:367, 1987 which is herein incorporated by reference for material at least related to hybridization of nucleic acids). A preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further, depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as

homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

183. Another way to define selective hybridization is by looking at the amount (percentage) of one of the nucleic acids bound to the other nucleic acid. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the limiting nucleic acid is bound to the non-limiting nucleic acid. Typically, the non-limiting primer is in for example, 10 or 100 or 1000 fold excess. This type of assay can be performed at under conditions where both the limiting and non-limiting primer are for example, 10 fold or 100 fold or 1000 fold below their k_d, or where only one of the nucleic acid molecules is 10 fold or 100 fold or 1000 fold or where one or both nucleic acid molecules are above their k_d.

184. Another way to define selective hybridization is by looking at the percentage of primer that gets enzymatically manipulated under conditions where hybridization is required to promote the desired enzymatic manipulation. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer is enzymatically manipulated under conditions which promote the enzymatic manipulation, for example if the enzymatic manipulation is DNA extension, then selective hybridization conditions would be when at least about 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer molecules are extended. Preferred conditions also include those suggested by the manufacturer or indicated in the art as being appropriate for the enzyme performing the manipulation.

185. Just as with homology, it is understood that there are a variety of methods herein disclosed for determining the level of hybridization between two nucleic acid molecules. It is understood that these methods and conditions may provide different percentages of hybridization between two nucleic acid molecules, but unless otherwise indicated meeting the parameters of any of the methods would be sufficient. For example if 80% hybridization was required and as long as hybridization occurs within the required parameters in any one of these methods it is considered disclosed herein.

186. It is understood that those of skill in the art understand that if a composition or method meets any one of these criteria for determining hybridization either collectively or singly it is a composition or method that is disclosed herein.

3. Nucleic acids

187. There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example DHR96 or variants or fragments thereof, as well as various functional nucleic acids. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantagous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

a) Nucleotides and related molecules

188. A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. An non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

189. A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl (.psi.), hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and

190. 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and

7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found for example in U.S. Pat. No. 3,687,808, Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine can increase the stability of duplex formation. Often time base modifications can be combined with for example a sugar modifcation, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications. Each of these patents is herein incorporated by reference.

191. Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10}, alkyl or C_2 to C_{10} alkenyl and alkynyl. 2' sugar modiifcations also include but are not limited to $-O[(CH_2)_n O]_m CH_3$, $-O(CH_2)_n OCH_3$, $-O(CH_2)_n NH_2$, $-O(CH_2)_n CH_3$, $-O(CH_2)_n -ONH_2$, and $-O(CH_2)_nON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

192. Other modifications at the 2' position include but are not limted to: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, $SO_2 CH_3$, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs may also have sugar mimetics such as

cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

193. Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. Numerous United States patents teach how to make and use nucleotides containing modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

194. It is understood that nucleotide analogs need only contain a single modification, but may also contain multiple modifications within one of the moieties or between different moieties.

195. Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

196. Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a

standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones;formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

197. It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by for example an amide type linkage (aminoethylglycine) (PNA). United States patents 5,539,082; 5,714,331;and 5,719,262 teach how to make and use PNA molecules, each of which is herein incorporated by reference. (See also Nielsen et al., Science, 1991, 254, 1497-1500).

198. It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,

199. 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Let., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane

acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937. Numerous United States patents teach the preparation of such conjugates and include, but are not limited to U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

200. A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

201. A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

b) Sequences

202. There are a variety of sequences related to the DHR96 gene, and these sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

203. One particular sequence set forth in SEQ ID NO:7 and having Genbank accession number NM_079769 is used herein, as an example, to exemplify the disclosed compositions and methods. It is understood that the description related to this sequence is applicable to any sequence related to DHR96 or any other sequences disclosed herein, unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences (i.e. sequences of DHR96 or variants or fragments thereof). Primers and/or

probes can be designed for any DHR96 sequence given the information disclosed herein and known in the art.

c) **Primers and probes**

204. Disclosed are compositions including primers and probes, which are capable of interacting with the genes disclosed herein. In certain embodiments the primers are used to support DNA amplification reactions. Typically the primers will be capable of being extended in a sequence specific manner. Extension of a primer in a sequence specific manner includes any methods wherein the sequence and/or composition of the nucleic acid molecule to which the primer is hybridized or otherwise associated directs or influences the composition or sequence of the product produced by the extension of the primer. Extension of the primer in a sequence specific manner therefore includes, but is not limited to, PCR, DNA sequencing, DNA extension, DNA polymerization, RNA transcription, or reverse transcription. Techniques and conditions that amplify the primer in a sequence specific manner are preferred. In certain embodiments the primers are used for the DNA amplification reactions, such as PCR or direct sequencing. It is understood that in certain embodiments the primers can also be extended using non-enzymatic techniques, where for example, the nucleotides or oligonucleotides used to extend the primer are modified such that they will chemically react to extend the primer in a sequence specific manner. Typically the disclosed primers hybridize with the nucleic acid or region of the nucleic acid or they hybridize with the complement of the nucleic acid or complement of a region of the nucleic acid.

4. Delivery of the compositions to cells

205. There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991)Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modifed to specifically function with large DNA molecules.

Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

a) Nucleic acid based delivery systems

206. The term "transgene" is used herein to describe genetic material which is artificially inserted into the genome of an invertebrate cell. The transgene encodes a product that, when expressed in embryos, gives rise to a specific phenotype. A transgene can encode a transcription factor or mimetic thereof having the desired result. A recombinant DNA molecule or vector containing a heterologous protein gene expression unit can be used to transfect invertebrate cells (United States Patents 4,670,388 and 5,550,043, herein incorporated by reference in their entirety.) A gene expression unit can contain a DNA coding sequence for a selected protein or for a derivative thereof. Such derivatives can be obtained by manipulation of the gene sequence using traditional genetic engineering techniques, e.g., mutagenesis, restriction endonuclease treatment, ligation of other gene sequences including synthetic sequences and the like (T. Maniatis et al, Molecular Cloning, A Laboratory Manual., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982).

207. Expression of the transgene can be targeted to occur in a non-adult stage of the animal, the transgene can be stably integrated into the genome of the animal in a manner such that its expression is controlled both spatially and temporally to the desired cell type and the correct developmental stage, i.e. to expression in embryonic neuroblasts. Specifically, the subject transgene can stably integrated into the genome of the animal under the control of a promoter that provides for expression. The transgene may be under the control of any convenient promoter that provides for this requisite spatial and temporal expression pattern, where the promoter can be endogenous or exogenous. A suitable promoter is the promoter located in the Drosophila melanogaster genome at position 86E1-3.

208. Another suitable promoter of the Drosophila origin includes the Drosophila metallothionein promoter (Lastowski-Perry et al, J. Biol. Chem., 260:1527, 1985). This inducible promoter directs high-level transcription of the gene in the presence of metals, e.g., CuSO4. Use of the Drosophila metallothionein promoter results in the expression system of the invention retaining full regulation even at very high copy number. This is in direct contrast to the use of the mammalian metallothionein promoter in mammalian cells in which the regulatory effect of the metal is diminished as copy number increases. In the Drosophila expression system, this retained inducibility effect increases expression of the gene product in the Drosophila cell at high copy number.

209. The Drosophila actin 5C gene promoter (B. J. Bond et al, Mol. Cell. Biol., 6: 2080, 1986) is also a desirable promoter sequence. The actin 5C promoter is a constitutive promoter and does not require addition of metal. Therefore, it is better-suited for use in a large scale production system, like a perfusion system, than is the Drosophila metallothionein promoter. An additional advantage is that the absence of a high concentration of copper in the media maintains the cells in a healthier state for longer periods of time.

210. Examples of other known Drosophila promoters include, e.g., the inducible heatshock (Hsp70) and COPIA LTR promoters. The SV40 early promoter gives lower levels of expression than the Drosophila metallothionein promoter.

211. The transgene may be integrated into the fly genome in a manner that provides for direct or indirect expression activation by the promoter, i.e. in a manner that provides for either cis or trans activation of gene expression by the promoter. In other words, expression of the transgene may be mediated directly by the promoter, or through one or more transactivating agents. Where the transgene is under direct control of the promoter, i.e. the promoter regulates expression of the transgene in a cis fashion, the transgene is stably integrated into the genome of the fly at a site sufficiently proximal to the promoter and in frame with the promoter such that cis regulation by the promoter occurs.

212. In other embodiments where expression of the transgene is indirectly mediated by the endogenous promoter, the promoter controls expression of the transgene through one or more transactivating agents, usually one transactivating agent, i.e. an agent whose expression is directly controlled by the promoter and which binds to the region of the transgene in a manner sufficient to turn on expression of the transgene. Any convenient transactivator may be employed. The GAL4 transactivator system an example of such a system.

213. The GAL4 encoding sequence can be stably integrated into the genome of the animal in a manner such that it is operatively linked to the endogenous promoter that provides expression in the appropriate location. The GAL4 system consists of the yeast transcriptional activator GAL4 and its target the upstream activating sequence (UAS) located within the P-element. Initially, GAL4 and UAS are in separate lines. The UAS is mobilized to generate new UAS insertion lines which remain silent until a source of GAL4 is made available. Under the control of a promoter, the expression of GAL4 is directed in a particular pattern. Specialized promoters can be used to drive expression of GAL4 in tissue and cell specific manners. The GAL4 containing line is then crossed to the UAS containing line. The UAS in the presence of

GAL4 directs the expression of any genes adjacent to its insertion site. When the insertion site is located upstream from the coding region over-or ectopic expression occurs.

214. Flies of line 31-1 (also referred to as 1822), as disclosed in Brand & Perrimon, Development (1993) 118: 401-415 express GAL4 in this manner, and are known to those of skill in the art. The transgene is stably integrated into a different location of the genome, generally a random location in the genome, where the transgene is operatively linked to an upstream activator sequence, i.e. UAS sequence, to which GAL4 binds and turns on expression of the transgene. Transgenic flies having a UAS: GAL4 transactivation system are known to those of skill in the art and are described in Brand & Perrimon, Development (1993) 118: 401-415; and Phelps & Brand, Methods (April 1998) 14:367-379.

215. A desirable gene expression unit or expression vector for the protein of interest cal also be constructed by fusing the protein coding sequence to a desirable signal sequence. The signal sequence functions to direct secretion of the protein from the host cell. Such a signal sequence may be derived from the sequence of tissue plasminogen activator (tPA). Other available signal sequences include, e.g., those derived from Herpes Simplex virus gene HSV-I gD (Lasky et al, Science, 233:209-212 1986).

216. The DNA coding sequence can also be followed by a polyadenylation (poly A) region, such as an SV40 early poly A region. The poly A region which functions in the polyadenylation of RNA transcripts appears to play a role in stabilizing transcription. A similar poly A region can be derived from a variety of genes in which it is naturally present. This region can also be modified to alter its sequence provided that polyadenylation and transcript stabilization functions are not significantly adversely affected.

217. The recombinant DNA molecule may also carry a genetic selection marker, as well as the protein gene functions. The selection marker can be any gene or genes which cause a readily detectable phenotypic change in a transfected host cell. Such phenotypic change can be, for example, drug resistance, such as the gene for hygromycin B resistance (i.e., hygromycin B phosphotransferase).

218. Alternatively, a selection system using the drug methotrexate, and prokaryotic dihydrofolate reductase (DHFR) gene, can be used with Invertebrate cells. The endogenous eukaryotic DHFR of the cells is inhibited by methotrexate. Therefore, by transfecting the cells with a plasmid containing the prokaryotic DHFR which is insensitive to methotrexate and selecting with methotrexate, only cells transfected with and expressing the prokaryotic DHFR will survive. Unlike methotrexate, selection of transformed mammalian and bacterial cells, in the

Drosophila system, methotrexate can be used to initially high-copy number transfectants. Only cells which have incorporated the protective prokaryotic DHFR gene will survive. Concomitantly, these cells have the gene expression unit of interest.

219. The subject transgenic flies can be prepared using any convenient protocol that provides for stable integration of the transgene into the fly genome in a manner sufficient to provide for the requisite spatial and temporal expression of the transgene, i.e. in embryonic neuroblasts. A number of different strategies can be employed to obtain the integration of the transgene with the requisite expression pattern. Generally, methods of producing the subject transgenic flies involve stable integration of the transgene into the fly genome. Stable integration is achieved by first introducing the transgene into a cell or cells of the fly, e.g. a fly embryo. The transgene is generally present on a suitable vector, such as a plasmid. Transgene introduction may be accomplished using any convenient protocol, where suitable protocols include: electroporation, microinjection, vesicle delivery, e.g. liposome delivery vehicles, and the like. Following introduction of the transgene into the cell(s), the transgene is stably integrated into the genome of the cell. Stable integration may be either site specific or random, but is generally random.

220. Where integration is random, the transgene is typically integrated with the use of transposase. In such embodiments, the transgene can be introduced into the cell(s) within a vector that includes the requisite P element, terminal 31 base pair inverted repeats. Where the cell into which the transgene is to be integrated does not comprise an endogenous transposase, a vector encoding a transposase can also be introduced into the cell, e.g. a helper plasmid comprising a transposase gene, such as pTURBO (Steller & Pirrotta, Mol. Cell. Biol. 6:1640-1649, 1986). Methods of random integration of transgenes into the genome of a target Drosophila melanogaster cell(s) are disclosed in U.S. Pat. No. 4,670,388, the disclosure of which is herein incorporated by reference.

221. Transcription and expression of the heterologous protein coding sequences can be monitored. For example, Southern blot analysis can be used to determine copy number of the gp120 gene. Northern blot analysis provides information regarding the size of the transcribed gene sequence. The level of transcription can also be quantitated. Expression of the selected protein in the recombinant cells can be further verified through Western blot analysis, for example.

222. In those embodiments in which the transgene is stably integrated in a random fashion into the fly genome, means are also provided for selectively expressing the transgene at

the appropriate time during development of the fly. In other words, means are provided for obtaining targeted expression of the transgene. To obtain the desired targeted expression of the randomly integrated transgene, integration of particular promoter upstream of the transgene, as a single unit in the P element vector may be employed. Alternatively, a transactivator that mediates expression of the transgene may be employed. Of particular interest is the GAL4 system described in Brand & Perrimon, Development (1993) 118: 401-415; and Phelps & Brand, Methods (April 1998) 14:367-379.

223. In one embodiment, the subject transgenic flies are produced by: (1) generating two separate lines of transgenic flies: (a) a first line that expresses GAL4; and (b) a second line in which the transgene is stably integrated into the cell genome and is fused to a UAS domain; (2) crossing the two lines; and (3) screening the progeny for the desired phenotype, i.e. adult onset neurodegeneration. Each of the above steps are well known to those of skill in the art (Brand & Perrimon, Development 118: 401-415, 1993; and Phelps & Brand, Methods 14:367-379, April 1998.)

b) Non-nucleic acid based systems

224. The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example in vivo or in vitro.

225. Thus, the compositions can comprise, in addition to the disclosed compositions or vectors for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

226. In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of

the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the disclosed nucleic acid or vector can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

227. The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). These techniques can be used for a variety of other speciifc cell types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated

endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

228. Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral intergration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

229. Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

c) In vivo/ex vivo

230. As described above, the compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the subject=s cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

231. If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

5. Peptides

a) Protein variants

232. As discussed herein there are numerous variants of the DHR96 protein that are known and herein contemplated. In addition, to the known functional DHR96 strain variants there are derivatives of the DHR96 protein which also function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and

in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

233. TABLE 1:Amino Acid Abbreviations

Amino Acid	Abbreviations
alanine	AlaA
allosoleucine	AIle
arginine	ArgR
asparagine	AsnN
aspartic acid	AspD
cysteine	CysC
glutamic acid	GluE
glutamine	GlnK
glycine	GlyG
histidine	HisH
isolelucine	IleI

Amino Acid	Abbreviations
leucine	LeuL
lysine	LysK
phenylalanine	PheF
proline	ProP
pyroglutamic acidp	Glu
serine	SerS
threonine	ThrT
tyrosine	TyrY
tryptophan	TrpW
valine	ValV

TABLE 2:Amino Acid Substitutions
Original Residue Exemplary Conservative Substitutions, others are known in the art.
Alaser
Arglys, gln
Asngln; his
Aspglu
Cysser
Glnasn, lys
Gluasp
Glypro
Hisasn;gln
Ileleu; val
Leuile; val
Lysarg; gln;
MetLeu; ile
Phemet; leu; tyr
Serthr
Thrser
Trptyr
Tyrtrp; phe
Valile; leu

234. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

235. For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

236. Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

237. Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

238. It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:8 sets forth a particular sequence of DHR96 cDNA and SEQ ID NO:7 sets forth a particular sequence of a DHR96 protein. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

239. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology

alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

240. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

241. It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

242. As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. For example, one of the many nucleic acid sequences that can encode the protein sequence set forth in SEQ ID NO:7 is set forth in SEQ ID NO:8. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular organism from which that protein arises is also known and herein disclosed and described.

243. It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids or amino acids which have a different functional substituent then the amino acids shown in Table 1 and Table 2. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way (Thorson et al., Methods in Molec. Biol. 77:43-

73 (1991), Zoller, Current Opinion in Biotechnology, 3:348-354 (1992); Ibba, Biotechnology & Genetic Engineerring Reviews 13:197-216 (1995), Cahill et al., TIBS, 14(10):400-403 (1989); Benner, TIB Tech, 12:158-163 (1994); Ibba and Hennecke, Bio/technology, 12:678-682 (1994) all of which are herein incorporated by reference at least for material related to amino acid analogs).

244. Molecules can be produced that resemble peptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH_2NH--, --CH_2S--, --CH_2--CH_2 --, --CH=CH-- (cis and trans), --$COCH_2$ --, --CH(OH)CH_2--, and --CHH_2SO—(These and others can be found in Spatola, A. F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, Trends Pharm Sci (1980) pp. 463-468; Hudson, D. et al., Int J Pept Prot Res 14:177-185 (1979) (--CH_2NH--, CH_2CH_2--); Spatola et al. Life Sci 38:1243-1249 (1986) (--CH H_2--S); Hann J. Chem. Soc Perkin Trans. I 307-314 (1982) (--CH--CH--, cis and trans); Almquist et al. J. Med. Chem. 23:1392-1398 (1980) (--$COCH_2$--); Jennings-White et al. Tetrahedron Lett 23:2533 (1982) (--$COCH_2$--); Szelke et al. European Appln, EP 45665 CA (1982): 97:39405 (1982) (--CH(OH)CH_2--); Holladay et al. Tetrahedron. Lett 24:4401-4404 (1983) (--C(OH)CH_2--); and Hruby Life Sci 31:189-199 (1982) (--CH_2--S--); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is --CH_2NH--. It is understood that peptide analogs can have more than one atom between the bond atoms, such as b-alanine, g-aminobutyric acid, and the like.

245. Amino acid analogs and analogs and peptide analogs often have enhanced or desirable properties, such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others.

246. D-amino acids can be used to generate more stable peptides, because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations. (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference).

6. Pharmaceutical carriers/Delivery of pharamceutical products

247. As described above, the compositions can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

248. The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, including topical intranasal administration or administration by inhalant. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

249. Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

250. The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J.

Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

a) Pharmaceutically Acceptable Carriers

251. The compositions, including antibodies, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

252. Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that

certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

253. Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

254. Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

255. The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

256. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

257. Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

258. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable..

259. Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

b) Therapeutic Uses

260. Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, guidance in selecting appropriate doses for antibodies can be found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical daily dosage of the antibody used alone might range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

7. Chips and micro arrays

261. Disclosed are chips where at least one address is the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at least one address is the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein.

262. Also disclosed are chips where at least one address is a variant of the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also

disclosed are chips where at least one address is a variant of the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein.

8. Computer readable mediums

263. It is understood that the disclosed nucleic acids and proteins can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed. Specifically contemplated herein is the display of these sequences on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks, or other computer readable mediums. Also disclosed are the binary code representations of the disclosed sequences. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded, stored, or saved.

264. Disclosed are computer readable mediums comprising the sequences and information regarding the sequences set forth herein. Also disclosed are computer readable mediums comprising the sequences and information regarding the sequences set forth herein wherein the sequences do not include SEQ ID Nos: 37, 38, 39, 40, 41, and 42.

9. Kits

265. Disclosed herein are kits that are drawn to reagents that can be used in practicing the methods disclosed herein. The kits can include any reagent or combination of reagent discussed herein or that would be understood to be required or beneficial in the practice of the disclosed methods. For example, the kits could include primers to perform the amplification reactions discussed in certain embodiments of the methods, as well as the buffers and enzymes required to use the primers as intended.

D. Methods of making the compositions

266. The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

1. Nucleic acid synthesis

267. For example, the nucleic acids, such as, the oligonucleotides to be used as primers can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic

digestion followed by nucleotide fragment isolation (see for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al.*, *Ann. Rev. Biochem.* 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* 5:3-7 (1994).

2. Peptide synthesis

268. One method of producing the disclosed proteins, such as SEQ ID NO:23, is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the disclosed proteins, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY (which is herein incorporated by reference at least for material related to peptide synthesis). Alternatively, the peptide or polypeptide is independently synthesized *in vivo* as described herein. Once isolated, these independent peptides or polypeptides may be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

269. For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large

peptides or polypeptides from shorter peptide fragments. This method consists of a two step chemical reaction (Dawson et al. Synthesis of Proteins by Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide--thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolini M et al. (1992) FEBS Lett. 307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al., Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

270. Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

3. Processes for making the compositions

271. Disclosed are processes for making the compositions as well as making the intermediates leading to the compositions. For example, disclosed are nucleic acids and proteins in SEQ ID NOs:1-60. There are a variety of methods that can be used for making these compositions, such as synthetic chemical methods and standard molecular biology methods. It is understood that the methods of making these and the other disclosed compositions are specifically disclosed.

272. Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid comprising the sequence set forth herein and a sequence controlling the expression of the nucleic acid.

273. Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence having 80% identity to a sequence set forth in herein, and a sequence controlling the expression of the nucleic acid.

274. Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence that hybridizes under stringent hybridization conditions to a sequence set forth herein and a sequence controlling the expression of the nucleic acid.

275. Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence encoding a peptide set forth in SEQ ID NO:7 and a sequence controlling an expression of the nucleic acid molecule.

276. Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence encoding a peptide having 80% identity to a peptide set forth in herein and a sequence controlling an expression of the nucleic acid molecule.

277. Disclosed are nucleic acids produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence encoding a peptide having 80% identity to a peptide set forth in herein, wherein any change from the herein are conservative changes and a sequence controlling an expression of the nucleic acid molecule.

278. Disclosed are cells produced by the process of transforming the cell with any of the disclosed nucleic acids. Disclosed are cells produced by the process of transforming the cell with any of the non-naturally occurring disclosed nucleic acids.

279. Disclosed are any of the disclosed peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the non-naturally occurring disclosed peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the disclosed peptides produced by the process of expressing any of the non-naturally disclosed nucleic acids.

280. Disclosed are animals and invertebrates produced by the process of transfecting a cell within the animal or invertebrate with any of the nucleic acid molecules disclosed herein. Disclosed are animals or invertebrates produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the animal is a mammal invertebrate is an insect, such as drosophila. Also disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate.

281. Also disclose are animals produced by the process of adding to the animal any of the cells disclosed herein.

E. Methods of using the compositions

1. Methods of using the compositions as research tools

282. The disclosed compositions can be used in a variety of ways as research tools. For example, the disclosed compositions, such as molecules disclosed herein can be used to

study the interactions between the molecules, and for example, their ligands or other compounds, by for example acting as inhibitors of binding.

283. The compositions can be used for example as targets in combinatorial chemistry protocols or other screening protocols to isolate molecules that possess desired functional properties related to inhibiting DHR96 activity, for example.

284. The disclosed compositions can be used as discussed herein as either reagents in micro arrays or as reagents to probe or analyze existing microarrays. The disclosed compositions can be used in any known method for isolating or identifying single nucleotide polymorphisms. The compositions can also be used in any method for determining allelic analysis of for example, DHR96, particularly allelic analysis as it relates to xenobiotic pathway functions. The compositions can also be used in any known method of screening assays, related to chip/micro arrays. The compositions can also be used in any known way of using the computer readable embodiments of the disclosed compositions, for example, to study relatedness or to perform molecular modeling analysis related to the disclosed compositions.

F. Examples

285. The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1 The DHR96 nuclear receptor is required for xenobiotic responses in *Drosophila*

a) Materials and Methods

(1) Construction of the DHR96 targeting fragment

286. A 7.55 kb DNA fragment that contains a mutated version of the Drosophila melanogaster DHR96 gene was generated by introducing two deletions: (1) deleting sequences harboring the start site (26 bp) and (2) deleting the fourth exon and intron (331 bp) from the wild type sequence. In addition, a recognition site for the restriction enzyme I-Sce I was inserted into the center (cuts between position 3699 and 3700) of the 7.55 kb fragment (see fig. M1). To obtain a genomic clone DNA of the P1 clone 26-95 that harbored the complete DHR96 gene was

isolated (provided by BDGP: http://www.fruitfly.org/). The assembly of the 7.55 kb targeting sequence was achieved by fusing three fragments:

(a) Fragment 1 A 1.958 kb Apa I-Hind III fragment

287. This was isolated by cutting P1 26-95 with Hind III and isolating a 6.599 kb Hind III fragment, which then was cut with Apa I and Sgr AI. The 1.958 kb Apa I – Hind III fragment was cloned into Litmus 38 (New England BioLabs) (cut with Apa I and Hind III).

(b) Fragment 2 A 4.325 kb fragment

288. This fragment contains the actual mutations and forms the core of the targeting construct. It was generated by using three pairs of PCR primers (for sequences, see oligos): (I) FAPA96 and R96EX3Sce, (II) F96Int3Sce and R96Int3, (III) F96Ex5Int3 and R96EndHind. The P1 26-95 genomic clone served as a template. Primer pair (I) produced a 1724 bp fragment, primer pair (II) a 993 bp fragment and primer pair (III) a 1650 bp fragment. The 993 bp and the 1650 bp fragments were fused in a PCR reaction using the primers F96Int3Sce and R96EndHind, generating a 2.62 kb fragment. Likewise, the 1724 bp and the 993 bp fragments were fused using the FAPA96 and R96Int3 primers to form a 2.70 kb fragment. In a final step, the 2.70 and the 2.62 kb fragments were fused using the primers FAPA96 and R96EndHind to form the aforementioned 4.325 kb fragment, which was cloned into PCR TOPO 2.1 (Invitrogen).

(c) Fragment 3 A 1.86 kb PCR fragment

289. Fragment 3 was generated using the primers F96Xma and R96SpeBgl, with the P1 26-95 clone as a template. The fragment was eluted and cut directly with Xma I and Spe I.

290. The 1.86 kb PCR fragment was cloned into the PCR Topo 2.1 vector (Invitrogen) containing the 4.325 kb, which was cut with Xma I and Spe I. The resulting clone was cut with Apa I and Spe I and fused to the 1.958 kb fragment, which had been previously isolated from Litmus 38 (New England Biolabs) with Apa I and Spe I. The resulting clone is the 7.55 kb targeting fragment. A sequence printout and annotation of this fragment is included (SEQ ID NO:37).

(2) Construction of the hs-Gal4-DHR96 fusion gene

291. A fusion of the Gal4 DNA binding domain (amino acids 1 to 147) and the DHR96 hinge region and ligand binding domain (LBD) (amino acids 99 to 723) was generated to create a Gal4-LBD fusion protein. Two PCR fragments were generated: (I) a 475 bp fragment using the primers FGALXB and RGAL96 and a Gal4 containing plasmid as a template. (II) F96BEG and R96/936 generate a 372 bp fragment from pLF20N, which contains the DHR96 cDNA (Fisk and Thummel, 1995). Fragments (I) and (II) possess a 15 bp overlap that was then

utilized to fuse them by PCR. The resulting 832 bp fragment was cut with Xba I and Age I and cloned into pLF20N, which had been cut with the same enzymes to remove the DHR96 DNA-binding domain. The resulting plasmid is termed pGAL96 . To obtain the final transformation vector, the Gal4-DHR96 fusion gene was isolated from pGAL96 with Not I and Nhe I and ligated to pCASPER hs-act cut with Xba I and Not I (SEQ ID NO:38, (see Seq 2 for the sequence of the insert in this vector, encoding the Gal4-LBD fusion).

(3) Construction of the hs-DHR96 RNAi vector

292. An inverted repeat sequence that corresponds to a part of the coding region for the DHR96 ligand-binding domain (each repeat corresponds to nucleotides 1444-2371 of the DHR96 plasmid pLF20N; Fisk and Thummel, 1995) was generated. The repeats are separated by a unique spacer region of 101 bp that corresponds to nucleotides 2372-2472 of the same DHR96 cDNA. Two primer pairs were used: (I) F96Xbai and R96BspE1 and (II) F96Xbai and R96BspE2. Both fragments were cut with Bsp EI and ligated. The ligated fragment was purified and cut with Xba I and cloned into Litmus 28 (New England Biolabs) cut with Xba I. After the cloned fragment (1956 bp) was verified by restriction analysis, it was excised with Xba I and inserted into pCasper hs-act cut with Xba I.

(4) Construction of the hs-DHR96 vector and fly transformation

293. This vector produces wild type DHR96 protein under the control of an hsp70 promoter in a transgenic animal. A full length cDNA was excised from the plasmid pLF20N with the restriction enzymes Not I and NheI and cloned it into pCasper hs-act vector cut with Not I and Xba I. Transformant flies were isolated using standard methods (Rubin GM, Spradling AC. Genetic transformation of Drosophila with transposable element vectors. Science. 1982 Oct 22;218(4570):348-53).

(5) Construction of pET24c-DHR96

294. To generate antibodies, DHR96 antigen was produced from a 1.8 kb EcoRV fragment (597 amino acids), which includes most of the cDNA, but excludes the DNA binding domain. The 1.8 kb Eco RV fragment was isolated from pLF20, a plasmid that contains a full length DHR96 cDNA (pLF20 differs from pLF20N in the following: pLF20 was cut with HindIII, filled in, and religated to create a unique Nhe I site. The new plasmid was termed pLF20N). pET24c (Novagen) was cut with Bam HI and Xho I and blunt ends were generated by fill-in, and subsequently the Eco RV fragment was cloned into this vector. Orientation was tested using restriction analysis. A sequence printout of this clone is included (SEQ ID NO:39Seq. 3).

(6) Construction of pMAL-DHR96

295. To purify antisera, soluble DHR96 protein was produced by fusing the original antigen to the Maltose-binding protein. To subclone the Eco RV fragment of DHR96 (the original antigen coding section) into pMAL-c2X (New England Biolab), a fragment from pET24c-DHR96 was PCR amplified by using the primer pair F96ANhe and R96AHind. The fragment was cut directly with Nhe I and HindIII and cloned into pMAL-c2X cut with Xba I and HindIII.

(7) Oligonucleotides

Oligonucleotides

SEQ ID NO:40	F96Xma	5'-GAGAGATGTGCTTCGTTAAAGCATCAACCC
SEQ ID NO:41	R96SpeBgl	5'-GGACTAGTAGATCTAGAGGATTCTACAAATGTCCAGTGTCTCCC
SEQ ID NO:42	R96Int3	5'-CCATTATTATCGCCATAATCGTAAAGG
SEQ ID NO:43	R96EX3SCE	5'-ATTACCCTGTTATCCCTAGCGGGTTACCTTAATGCGATCATCGCCC
SEQ ID NO:44	R96endhind	5'-GGAAAGCTTTTCCTGCTGATCAATAATACC
SEQ ID NO:45	FAPA96	5'-TGGGCCCATCACTTGCTTGTAACCGCCGAAGAACTGCGCGG
SEQ ID NO:46	F96INT3SCE	5' CGCTAGGGATAACAGGGTAATAACAGTCCACGGTATTAGCCTATAGG
SEQ ID NO:47	F96EX5Int3	5' CGATTATGGCGATAATAATGGCCAAAGAGAACATGGGCAACATACGC
SEQ ID NO:48	FGALXB	5'-GAAGCAAGCCTCTAGAAAGATGAAGC
SEQ ID NO:49	RGAL96	5'-CGTGCCGTTCTCCATCGATACAGTCAACTGTCTTTGACC
SEQ ID NO:50	R96/936	5'-GCCTGGATAGTCGATCAAATGCG
SEQ ID NO:51	F96BEG	5'-ATGGAGAACGGCACGGATGC
SEQ ID NO:52	F96XBAi	5'-TACATTCTAGAGACCAACTACAACGACGAGCCCAGTCTGG
SEQ ID NO:53	R96BspE1	5'-CATTCATCCGGACATTAATTATGAACTTGTTCAGACGCTCC
SEQ ID NO:54	R96BspE2	5'-GGGCATCAACTCCGGAATTAAATGCCCGACACGCATCGG
SEQ ID NO:55	RPAXCRE-AN	5'-GTCTCACGACGTTTTGAACCCAGAAATCGAGCTCGCCCGGGG
SEQ ID NO:56	RPAXCRECO	5'-CACGAATTCCAAACTGTCTCACGACGTTTTGAACCC
SEQ ID NO:57	FPAXFSE-AN	5'-GAGAGCTAGCATGCCGGCTAGATCTCGAGATCGGCCGGCCTAGG
SEQ ID NO:58	FPAXPOLY	5'-GAACTGCAGCTCGAGAGCTAGCATGCCGGC
SEQ ID NO:59	F96ANhe	5'-GGAGATATACATATGGCTAGCATGACTGGTGG
SEQ ID NO:60	R96AHind	5'-TGCTCGAAGCTTCGCAGAAGATAATAGTAGG

(8) DHR96 gene targeting

296. The 7.55 kb genomic fragment containing a mutated DHR96 gene (see above) was inserted into the Drosophila genome as described (Rong YS, Golic KG. Gene targeting by homologous recombination in Drosophila. Science. 2000 Jun 16;288(5473):2013-8). w; [hsp70-FLP]4 [hsp70 I Sce I]2b Sco/S2 CyO females were crossed to w; [<(96TG GFP+> w+] males that carried the targeting fragment on the second chromosome. Larvae were heat shocked during the third larval instar to trigger targeting events in the germline of females. [hsp70-FLP]4 [hsp70 I Sce I]2b Sco/ [<(96TG GFP+> w+] females were then collected and crossed them to w; Ser1/TM6B, Tb males. 918 vials of such crosses (5 males and 10 females) were set up which generated approximately 150,000 flies that were screened for GFP+, but white-eyed individuals. These flies were crossed to w1118; Ly/TM6C Tb Sb, and stocks were subsequently established from a single chromosome. The DHR96E25 allele was isolated from one of these stocks.

(9) Reduction of the DHR96 targeted event to a single copy by I-CreI

297. Males carrying the tandem duplication allele (w1118/Y; DHR96E25/DHR96E25) were mated to v hsp70 CreI; Sb/TM6 females in mass. After 3 days at 25°C, the parental flies were removed and the progeny were heat-treated at 36°C for one hour to induce CreI recombinase. Males that eclosed were individually mated to w1118; Ly/TM6C females. One male progeny (w1118/Y; DHR96Cre reduced/TM6C) that had lost GFP expression (indicating a recombination event had occurred) was selected from each vial and individually mated to w1118; Ly/TM6C females to establish a stock containing the reduced allele (Rong and Golic 2002). Mutant strains were characterized by Southern blotting, PCR, and DNA sequencing using standard methods. The DHR9616A mutant stock was selected for further characterization.

(10) Tissue antibody stains

298. Wandering third instar larval tissues were dissected and fixed as previously described (Boyd, L., O'Toole, E. and Thummel, C.S. (1991). Patterns of E74A RNA and protein expression at the onset of metamorphosis in Drosophila. Development 112, 981-995). DHR96 protein was detected with anti-DHR96 antibodies diluted 1:100 and incubated overnight at 4 °C. Donkey anti-rabbit CY3 secondary antibodies (Jackson) were used at a 1:200 dilution as a secondary antibody. The stains were visualized on a Biorad confocal laser scanning microscope.

(11) Western blots analysis

299. Protein from adult flies was extracted by grinding flies in SDS sample buffer and boiling. The equivalent of approximately one adult fly was loaded in each lane of an 8% polyacrylamide gel, separated by electrophoresis and transferred to PVDF membrane. Ectopically expressed DHR96 protein was produced by heat-treating flies at 37.5 o C for 30 minutes followed by a three hour recovery at room temperature before the extraction procedure. DHR96 protein was detected by incubating the membrane first with a 1:500 dilution of anti-DHR96 affinity purified antibodies followed by a 1:1000 dilution of goat anti-rabbit HRP secondary antibody (Pierce). A supersignal chemiluminescence kit was used to develop the signal (Pierce).

(12) Toxicity assays

300. Adult flies were raised on standard cornmeal/agar food and starved overnight under humid conditions at 25 0 C before treatment with DDT. A DDT stock solution was prepared by dissolving crystalline DDT (Sigma) in 100% ethanol. Appropriate DDT dilutions were made by diluting the DDT stock with 5% sucrose and pipetting 275 µl of the solution onto a strip of Whatman filter paper inside a small glass scintillation vial. Twenty adult flies were placed in each vial which was plugged with cotton. Mortality was scored 10 hours later at room temperature. For each DDT concentration, three replicates, each of twenty adult flies, were used. For the time course assay, 100 ng/µl of DDT was used and mortality scored every hour for 10 hours.

b) Results

(1) DHR96 is closely related to known xenobiotic receptors

301. The phylogenetic relationship of DHR96 to other nuclear receptors was investigated for information related to function. When performing a BLASTP search, the closest homolog to DHR96 in vertebrates is the Vitamin D3 Receptor (VDR). The Pregnane X Receptor (PXR) as well as the Constitutively Androstane Receptor (CAR) comprise other high scoring homologs. (Fig. 1).

(2) DHR96 is expressed in the alimentary canal, the salivary glands and the fat body

302. Antibody stains of third instar larvae were used to analyze whether DHR96 would be expressed in tissues that function in detoxification. DHR96 antibodies strongly stain tissues of the alimentary canal (Fig. 2). In particular, the gastric caeca, the major site of absorption in Diptera, show a much stronger staining than the remainder of the midgut, which also plays a role

in nutrient absorption. Strong expression in the Malpighian tubules, the principal excretory organ in insects, was also observed. The excretory system maintains homeostasis, controlling salt levels and osmotic pressure, but is primarily responsible for the removal of harmful metabolites such as nitrogenous wastes derived from purine metabolism, or toxic compounds that were absorbed from the food. Outside the alimentary canal, strong staining in the salivary gland and the fat body were detected. The insect fat body is the functional equivalent of the mammalian liver, because it is the principal site of intermediary metabolism and detoxification. Taken together, the finding that DHR96 expression is tightly associated with tissues known to be involved in detoxification provides strong support for the proposal that DHR96 functions in a xenobiotic pathway.

(3) DHR96 function is dispensable under standard conditions

303. RNA interference (RNAi) and gene targeting were used to disrupt *DHR96* function because no existing mutants were available. The effects of *DHR96* RNAi were analyzed by generating transgenic lines that express snapback RNA under the control of a heat-inducible promoter. Three independent lines showed strong reduction of *DHR96* mRNA in northern blots when treated with a single heat-shock, but displayed no discernable phenotype. Using a variety of heat-shock regimens, e.g. longer single and double treatments or 12 hr repetitions, did not affect the outcome of this observation. These findings suggest that *DHR96* mRNA is not necessary for viability under standard conditions, indicating either that DHR96 protein is very stable or dispensable for survival.

304. Gene targeting (Rong, Y. S., and Golic, K. G. (2000). Science 288, 2013-2018) was used to generate mutations in *DHR96* because no deficiencies or P elements were known in this region of the genome. As a first step, the gene targeting procedure requires classical P-element transformation in order to generate transgenes that harbor the targeting sequence flanked by *FRT* sites. The targeting DNA is then mobilized and turned into a linear, recombinogenic molecule *in vivo* by activating the *FLP* recombinase and the endonuclease *I Sce I*. As a consequence of this targeting technique, which is based on an "ends-in" mechanism, the resulting mutation is basically a replacement of the original gene with a tandem duplication of two mutant copies (Fig. 3). Mutations were engineered in such a way that both copies would result in non-functional gene products. In particular, a region around the translation start site (25 bp), and the complete sequence of exon four was deleted, the downstream intron, and the splice acceptor site at exon 5 (together ~300 bp). These mutations should lead to a block in translation initiation as well as removal of most of the ligand binding domain of the receptor.

We constructed a targeting vector that contained two eye markers: *pax6-EGFP* and mini-*white*. Once mobilized by the FLP recombinase, the EGFP gene separates physically from the mini-*white* gene, which lies outside the FRT sites. Consequently, the subsequent strategy employed to identify potential targeting events is based on the presence of the EGFP marker and the simultaneous absence of the mini-*white* marker in the eye.

305. In a screen of ~150,000 flies, a total of 42 events were detected. Of these, 18 mapped to the third chromosome, which harbors the *DHR96* gene. At least one of the 18 events was identified as a targeting event in the *DHR96* gene, and we termed this allele $DHR96^{E25}$. To avoid problems that might arise from the truncated protein in the $DHR96^{E25}$ mutant, we decided to reduce the existing duplication to one mutant copy by utilizing the *I Cre I* site that was built into the targeting vector, essentially following the procedure described by (Rong, Y. et al., (2002) Genes Dev *16*, 1568-1581). This procedure yielded a new *DHR96* allele, $DHR96^{16A}$, which, based on sequence and western analysis, constitutes a protein null. Several lines of evidence suggest that these alleles represent specific targeting events in the *DHR96* gene. First, genomic Southern blots of animals homozygous for the targeting events displayed the predicted fragment patterns of a tandem duplication ($DHR96^{E25}$) or a reduced single copy ($DHR96^{16A}$). Second, northern analysis revealed the absence of the wild type mRNA in the mutant animals. Third, antibody stains and Western analysis show a strong reduction or absence of the DHR96 protein in $DHR96^{16A}$or $DHR96^{E25}$ flies (add fig for this). Fourth, Southern blot hybridization and sequencing of PCR products demonstrated that exon/intron 4 of wild type *DHR96* is absent in homozygous $DHR96^{16A}$ or $DHR96^{E25}$ animals.

306. Flies homozygous for $DHR96^{E25}$ or $DHR96^{16A}$ are viable and fertile when grown on standard cornmeal food. However, when placed on instant food (Carolina 424) in the absence of yeast, viability decreases to about 1%, whereas wild type flies do comparably well with a survival rate of ~35% compared to standard food. Interestingly, the addition of yeast restores viability to 100%. This suggests that either *DHR96* is required for the proper execution of certain nutritional pathways, or that $DHR96^{E25}$ larvae fail to neutralize toxic metabolites that are produced when animals are reared on nutritionally poor media. To test the possibility that *DHR96* mutants have a decreased tolerance for toxins, it was determined whether DHR96 is expressed in tissues that are known to play critical roles in the detoxification process.

(4) DHR96 mutants display reduced viability in the presence of DDT

307. As a test of *DHR96* acting in a xenobiotic pathway, *DHR96* mutants were tested for sensitivity to the pesticide DDT. Adult wild type flies (Canton S) and *DHR96*^{16A} were exposed or *DHR96*^{E25} flies to varying concentrations of DDT and recorded survival rates after a fixed time. The findings showed that *DHR96* mutants were more sensitive to DDT and died at lower concentrations of DDT compared to control animals (Fig. 4A). In addition, when challenged with a fixed concentration of DDT, *DHR96* homozygotes died more rapidly than wild type flies (Fig. 4B). Taken together, these results indicated that DHR96 is required for natural resistance levels to the pesticide DDT, and that DHR96 functions in a xenobiotic response pathway.

(5) Overexpression of DHR96 has no effect on viability

308. Most nuclear receptors cause lethality when overexpressed, indicating that these proteins do not require an obligatory ligand for some or even all of their functions. To analyze whether DHR96 would disrupt essential pathways and cause lethality when expressed ectopically, a transgenic line that harbored a full-length *DHR96* cDNA under the control of a heat-inducible promoter was produced. Western and Northern analysis showed that heat-treated larvae and flies carrying this construct generated at least 100 times more *DHR96* mRNA and protein than wild type flies lacking the transgene. Nevertheless, overexpression of this protein did not result in any visible effect, suggesting two possible scenarios: (I) DHR96 activity requires binding to a ligand or a protein partner, or (II) DHR96 target genes do not function in vital pathways, at least not under standard laboratory conditions. Naturally, both possibilities may be true. Microarray experiments were used to dissect how DHR96 might function on the molecular level.

c) Microarray experiments

309. As a first step toward identifying target genes regulated by DHR96, the protein was overexpressed in larvae and analyzed its effects on gene expression by microarray analyzed. Affymetrix oligonucleotide chips designed to detect ~13,200 genes (the majority in the fly genome) were used, the raw data with dCHIP (Li C, Wong WH. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc Natl Acad Sci U S A. 2001 Jan 2;98(1):31-6; Li, C., and Wong, W. H. (2001) Genome Biol 2, 0032.1-0032.11; http://www.dchip.org/) was analyzed, and filtering with Microsoft Access was performed. After rigorous filtering, only 71 genes remained that had a higher than 1.8-fold

change when compared to the controls. Interestingly, of the top 20 reduced genes, six are members of all four major detoxification gene families (Table 3), which comprise a total of 198 members in *Drosophila*. This represents a highly significant result ($p=2.8x10^{-27}$, based on χ^2), because the chances of picking 6 of these genes in a random sample of 20 genes are more than 20-fold lower than the observed number. Interestingly, no such concentration of genes encoding detoxifying enzymes exists on the list of induced genes, suggesting that DHR96 may repress these genes in the absence of suitable ligands.

2. Example 2

a) GAL4-DHR96/LBD experiments

310. To determine if DHR96 is activated by the pesticide DDT the methods disclosed herein can be used. Flies containing two different transgenes will be mated together allowing us to directly assay for DHR96 LBD activation in vivo (for detailed methods and description of vectors see: (Kozlova, T., and C.S. Thummel (2003) Methods to characterize Drosophila nuclear receptor activation and function in vivo. In: "Methods in Enzymology. Nuclear Receptors, Vol. 364 (Russell, D.W., and Mangelsdorf, D.J., eds.), Academic Press, New York, pp. 475-490.)). One transgene is under the control of a heat-inducible promoter and contains the GAL4 DNA binding domain fused to the DHR96 ligand binding domain . The second transgene contains a GAL4-dependent GFP or lacZ reporter gene (Kozlova, T., and C.S. Thummel (2003) Methods to characterize Drosophila nuclear receptor activation and function in vivo. In: "Methods in Enzymology. Nuclear Receptors, Vol. 364 (Russell, D.W., and Mangelsdorf, D.J., eds.), Academic Press, New York, pp. 475-490.)). Upon heat induction, GAL4-DHR96 LBD protein can bind to the UAS-GFP or UAS-lacZ reporter. In the absence of a ligand, the reporter will not be activated; however, in the presence of a ligand, the GAL4 DHR96 LBD protein can be switched into an active conformation and induce reporter gene expression (Kozlova, T., and C.S. Thummel (2003) Methods to characterize Drosophila nuclear receptor activation and function in vivo. In: "Methods in Enzymology. Nuclear Receptors, Vol. 364 (Russell, D.W., and Mangelsdorf, D.J., eds.), Academic Press, New York, pp. 475-490.); Kozlova, T. and Thummel, C.S. (2002). Spatial patterns of ecdysteroid receptor activation during the onset of Drosophila metamorphosis. Development 129, 1739-1750).

311. To determine if drugs, such as DDT, can activate the DHR96 GAL4-LBD construct, two developmental stages will be tested. First, organs from late third instar larvae that have both transgenes will be dissected and cultured in the presence of several different concentrations of drug and assayed for reporter gene expression. Second, if activation of the

GAL4-LBD construct by drug requires either ingestion of the toxin or contact with the cuticle of the fly, adults will be heat-shocked to induce the GAL4-LBD construct, placed in scintillation vials containing drug, as previously above in the toxicity assays, and assayed for induction of reporter gene expression in adult tissues. Changes in the activity of the reporter gene in the presence, but not the absence, of drug will be an indication that that compound is having a direct effect on the activity state of the DHR96 LBD.

312. Disclosed are systems that can identify ligands, such as hormones, for nuclear receptors, such as drosophila nuclear receptors. There are many members of the nuclear receptor superfamily for which there is no known ligand – the so called orphan nuclear receptors. It is desirable to link these receptors to a ligand if it exists.

313. One way of identifying ligands for nuclear receptors involves expressing a fusion of the GAL4 DNA binding domain to a nuclear receptor ligand binding domain (LBD), in combination with a GAL4-reponsive reporter gene. The fusion protein is inactive unless its hormone is present, allowing it to switch into an active conformation and turn on the GAL4-responisve reporter, such as a lacZ report giving a color readout. In one variation of this method, which has been widely exploited by pharma companies for high throughput screens, stably transfected tissue culture cells of different cell types are used for the cell background to perform the assay. One way to do this assay would be use every tissue in the animal as a context for screening for hormones, not just a tissue culture cell where the appropriate cofactors or partner transcription factors might be missing, because presumably every cell has a different molecular background.

314. One method used to get around this problem in mice is disclosed in WO 00/17334 for "Analysis of ligand activated nuclear receptors (in vivo)" by Solomon et al. (See also, Solomin, L., et al., (1998). Nature 395, 398-402). This system was designed for the mouse, because the GAL4 system of linking the GAL4 DBD to a particular LBD works poorly in mouse.

315. Disclosed herein is a system for drosophila for identifying ligands for nuclear receptors, where the GAL4 system works very well for driving tissue- and stage-specific ectopic gene expression. The system typically utilizes a heat-inducible promoter to widely express the GAL4-LBD fusion proteins, but any inducible promoter can be used. This allows monitoring of activation in all tissues both spatially and temporally. The pattern of lacZ expression in animals so transformed allows visualization of where and when a particular LBD is active during development, guiding one towards possible sources of hormone.

316. This has been used to show the patterns of GAL4-EcR and GAL4-USP activation during the onset of metamorphosis accurately reflect what would be expected for regulation of EcR/USP by its hormone, 20-hydroxyecdysone (Kozlova, T. and Thummel, C.S. (2002). Spatial patterns of ecdysteroid receptor activation during the onset of Drosophila metamorphosis. Development 129, 1739-1750). Spatial patterns of ecdysteroid receptor activation during the onset of Drosophila metamorphosis. Development 129, 1739-1750). This system has also been used to show that an orphan nuclear receptor, DHR38, is activated by a unique set of ecdysteroids in the animal (Baker, K. D., et al., (2003). The Drosophila orphan nuclear receptor DHR38 mediates an atypical ecdysteroid signaling pathway. Cell 113, 731-742).

317. Disclosed herein are hsp70-GAL4-LBD transformants for all 18 Drosophila nuclear receptors. The activation patterns of these constructs have been characterized during embryogenesis and the onset of metamorphosis. These constructs can be used with a UAS-GFP reporter to simplify the readout of activation, paving the way for compound screens.

318. These constructs can be used to screen compounds for ligand activity. For example, a collection of pesticides can be found in the Agro plate (see http://www.msdiscovery.com). Other plates can also be found at Micro Source Discovery, and are herein incorporated by reference at least for compound libraries and their contents. They also list plates of available collections of natural compounds.

319. Disclosed are methods for screening for the presence of xenobiotic receptor ligands using the constructs and methods disclosed herein, such as those for the GAL4-DHR96 fusions.

G. References

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H. Sequences

1. SEQ ID NO: 1 Accession No. NM_130611 Drosophila melanogaster CG16902-PA

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ORIGIN

2. SEQ ID NO: 2 Accession No. NM_130611 Drosophila melanogaster CG16902-PA

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2401 ggcggtggtt cggtatctgg cggcggagtg ggcggaccac tcacaccctc ctcggtggcg
2461 ccgcagaata acgaggaggc cgcccaactc ctgctctccc tgggacagac acgcatccag
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2641 caacagcagc agtggggcag cagcaactcc acgggtcttg gtggcgtagg cggcggcatg
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2941 tgcatcgagc agggcatggt gctgcaagcc gttcgcgagg atcgcatgcc gggcggtcgc
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4201 accacgctaa tgggccagcc gatagcgatg gagcagctga agctggacgt cgggcacatg
4261 gtggacaaga tgacccagat caccatcatg ttccggcgaa tcaagctcaa gatggaggag
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4441 ctggagagca tccaggagcg gtacgtccag gtgctgcgct cctacctgca aaactcctcg
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3. SEQ ID NO: 3 Accession No. NM_168775 Drosophila melanogaster ftz transcription factor 1 CG4059-PA

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4. SEQ ID NO: 4 Accession No. NM_168775 Drosophila melanogaster ftz transcription factor 1 CG4059-PA

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121 gccctccagt cccgctctgg ccgccggtgg caacagcagc aacaacgcgg ccagcggtag
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5. SEQ ID NO: 5 Accession No. NM_176123 Drosophila melanogaster Hormone receptor-like in 46 CG33183-PA

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6. SEQ ID NO: 6 Accession No. NM_176123 Drosophila melanogaster Hormone receptor-like in 46 CG33183-PA

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4261 ca

7. SEQ ID NO: 7 Accession No. NM_079769 Drosophila melanogaster Hormone receptor-like in 96 CG11783-PA

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8. SEQ ID NO: 8 Accession No. NM_079769 Drosophila melanogaster Hormone receptor-like in 96 CG11783-PA

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1381 tgcagtacca ctcgcccggt gtcggggagc agcccagtac atcgagtagc cacccccttgc
1441 cttacatagc caactcgccg gacttcgatc tgaagacctt catgcagacc aactacaacg
1501 acgagcccag tctggacagt gattttagca ttaactcaat cgaatcggtg ctatccgagg
1561 tgatccgcat tgagtaccag gccttcaata gcatacaaca agcggcatcg cgcgtaaagg
1621 aggagatgtc ctacggcact cagtctacgt acggtggatg caattcggct gcaaacaata
1681 gccagccgca cctgcagcaa cccatctgcg ccccatccac ccagcagttg gatcgcgagc
1741 taaacgaggc ggagcaaatg aagctgcggg agctgcgact ggccagcgag gctctttatg
1801 atcccgtgga cgaggacctc agcgccctga tgatgggcga tgatcgcatt aagcccgacg
1861 acactcgcca caacccaaag ctattgcagc tgatcaatct gacggcggtg gccatcaagc
1921 ggcttatcaa aatggccaag aagattacag cattccgtga catgtgccag gaggaccagg
1981 tggccctact caaaggtggc tgcacagaaa tgatgataat gcgctccgta atgatttacg
2041 acgacgatcg cgccgcctgg aaggtacccc ataccaaaga gaacatgggc aacatacgca
2101 ctgacctgct caagtttgcc gaaggcaata tctacgagga gcaccaaaag ttcatcacaa
2161 cgtttgacga gaagtggcgc atggacgaga acataatcct gatcatgtgt gccattgtcc
2221 ttttacctc ggctcgatcg cgagtgatac acaaagacgt gattagattg gaacagaatt
2281 cctactatta tcttctgcga agatatctgg agagtgttta ttctggctgt gaggcgagaa
2341 acgcgtttat caagctaatc caaaagattt cagatgtgga gcgtctgaac aagttcataa
2401 ttaatgtcta tttgaatgtt aacccatccc aggtggagcc cttgctgcgt gaaatattcg
2461 atttgaaaaa tcactagaca accgatgcgt gtcgggcatt taatgcctat gttgatgccc
2521 aatgatgaat ggtcaacaag ctgtagttgt tgttgttgtt gatgtctgtt ttatcttgtc
2581 gcttgtaatg ttagatttta atcgaatgtg attgttagat ttgcatatac tgcatagatt
2641 ttatatttct acatcaaaga gagcatattt aggataccaa gtgcaaagca acacaatcta
2701 tatgtaatgt acaccgttta cctagtttca aataaactag acgataatgc aataactaac

2761 ttggaagcgt gggttctgtg caaaaaggaa aaaagacaaa aaaaataaac tgactttgag
2821 aaccagtggt aa

9. SEQ ID NO: 9 Accession No. NM_057539 Drosophila melanogaster Hepatocyte nuclear factor 4 CG9310-PA

MMKHPQDLSVTDDQQLMKVNKVEKMEQELHDPESESHIMHADAL
ASAYPAASQPHSPIGLALSPNGGGLGLSNSSNQSSENFALCNGNGNAGSAGGGSASSG
SNNNNSMFSPNNNLSGSGSGTNSSQQQLQQQQQQSPTVCAICGDRATGKHYGASSCD
GCKGFFRRSVRKNHQYTCRFARNCVVDKDKRNQCRYCRLRKCFKAGMKKEAVQNERDR
ISCRRTSNDDPDPGNGLSVISLVKAENESRQSKAGAAMEPNINEDLSNKQFASINDVC
ESMKQQLLTLVEWAKQIPAFNELQLDDQVALLRAHAGEHLLLGLSRRSMHLKDVLLLS
NNCVITRHCPDPLVSPNLDISRIGARIIDELVTVMKDVGIDDTEFACIKALVFFDPNA
KGLNEPHRIKSLRHQILNNLEDYISDRQYESRGRFGEILLILPVLQSITWQMIEQIQF
AKIFGVAHIDSLLQEMLLGGELADNPLPLSPPNQSNDYQSPTHTGNMEGGNQVNSSLD
SLATSGGPGSHSLDLEVQHIQALIEANSADDSFRAYAASTAAAAAAAVSSSSSAPASV
APASISPPLNSPKSQHQHQQHATHQQQQESSYLDMPVKHYNGSRSGPLPTQHSPQRMH
PYQRAVASPVEVSSGGGGLGLRNPADITLNEYNRSEGSSAEELLRRTPLKIRAPEMLT

APAGYGTEPCRMTLKQEPETGY

10. SEQ ID NO: 10 Accession No. NM_057539 Drosophila melanogaster Hepatocyte nuclear factor 4 CG9310-PA

1 agttgaattc cagtgacgtt ggaagaaaca actgcaaaag gcaaaaacaa agacaatgtt
61 tataagctgt atattccgct ttgattgata taaatgaata tatgcagtgc gccagttata
121 caactgccct gcaaaagtca ctcattaaat aaaaaacgcc cgagatgaat ttcacagcgg
181 cggcaacaag tgcaataata gtaaaaaatc aaaagccaaa caacgaaatc tctcccaaaa
241 aaacgaagaa gcgtgtcgcg gtgccaaaaa gaaaacaaaa atagaaaaat acacaacaaa
301 ataatacgga gaaacgttaa ttataacgag ccacaaaatc gcataaagaa atcaacaagt
361 gtgtgtctgc cttttttcc atattcgctt tcattcatgc ggtcaactca acaataacaa
421 ctcaaaatag caacaacaac aataacaata tcaacaagag cagcagcagt cgctgataaa
481 agccctgcag ctaaaacaac aacaaaacaa caaagatagt tagaaagaac atcgtctggc
541 cattgagctt taattgccgg tcattacttc attactatgt gattggatct tcccgaccca
601 cttgtaaata aaaagtaaaa atactggtta tgaagcatga tgaagcatcc gcaggatctg
661 agtgtcacgg atgaccagca gttaatgaag gtgaacaagg tggagaagat ggagcaggag
721 ttgcacgacc ccgaatcgga gagccacata atgcacgcgg atgccctggc ctctgcctat
781 ccggctgcct cgcagcccca cagtccgatc ggcctcgccc tcagccccaa tggcggtggg
841 ctgggactga gcaacagtag caaccagagc agcgagaact ttgcgctctg caacggaaac
901 ggaaatgcgg gcagcgcagg aggcggaagt gccagcagtg gcagcaacaa caacaacagc
961 atgttctcac ccaacaacaa cttgagcgga agcggaagtg ggactaacag cagtcagcag
1021 caattgcagc agcaacaaca acagcaatca ccgacggtct gcgccatttg tggagatcgg
1081 gcgacgggca aacattatgg agcctccagc tgcgacggct gcaaaggatt cttcaggagg
1141 agtgtcagga aaaatcatca gtacacttgc agatttgcgc gaaactgcgt tgtggacaag
1201 gacaaacgga atcagtgccg ctactgccgg ctgaggaagt gcttcaaggc gggcatgaag
1261 aaggaggcgg tgcaaaacga gcgggatcgc attagctgcc gccgcacctc caatgacgac
1321 ccggatccgg gcaatgggct gtctgtgatt tccttggtta aggcggagaa tgagtcgcgt
1381 cagtcgaagg caggcgctgc catggagcca aacattaacg aggacctctc caacaagcag
1441 ttcgcgagca tcaacgatgt ctgcgagtcg atgaagcagc agctgctgac cctggtggaa
1501 tgggctaagc agattccggc ctttaacgag ctgcagctgg atgaccaggt ggcactgcta
1561 cgcgcccatg ctggcgagca tttgctcctc ggcctgtctc gtcgttcgat gcacttgaag
1621 gatgttctcc tgctgagcaa caattgtgtg atcacaaggc actgtccaga tccccttgtg
1681 tcgccgaatt tggacatctc ccggatcggc gcccgtatca tcgatgaact ggtgacggtc
1741 atgaaggatg tgggtatcga tgacactgaa ttcgcttgca tcaaggccct agtcttcttc
1801 gatcccaatg ccaagggtct taatgaaccg catcgcatca aatcgctacg gcatcagata

1861 ctcaataatc tcgaggacta catatcagat cggcaatacg agtcgcgcgg tcgctttggc
1921 gagattctgc tcatcctgcc ggttctgcag tctattacct ggcagatgat cgagcagatc
1981 cagtttgcca agatctttgg agtggcccac attgattcat tactgcagga aatgttgttg
2041 ggaggagagt tggccgacaa tcctctgccg ctatcgccgc ccaatcagtc aaatgactac
2101 cagagtccca cccacacagg caacatggag ggcggtaatc aagttaactc ctctctggac
2161 tcgctggcca cgtccggtgg tcctggctcg catagtctgg acctggaggt gcagcacatt
2221 caggctctta tcgaggcgaa cagtgcggat gattccttcc gggcctacgc ggccagcact
2281 gcagcggcag ccgctgcagc cgtctcgtcc tcctcctctg cacccgcatc cgttgctcca
2341 gcctcgatct ctcctccgct caacagcccc aagtcacaac atcaacatca gcaacatgcg
2401 acgcatcagc aacaacagga gagctcctac ttggacatgc ccgtcaagca ctacaatggc
2461 agtcggtccg gaccgctgcc aacacagcac agtccccaga ggatgcatcc ctaccaaaga
2521 gcagtcgcct cgccggtcga agtgtccagc gggggcggcg gattgggtct gcgcaatcct
2581 gccgatatta cgctcaacga gtacaaccgg agcgagggta gcagtgccga ggagctgctg
2641 cgacgaactc cactgaagat ccgggctccc gagatgctaa ccgcacccgc tggttatgga
2701 acggaaccct gtcgcatgac acttaaacag gagccagaga ctggttacta gaagaataac
2761 gaacggtgca atatgcagtt tgcaatagga cacccсttaa gcacacaacc catacacata
2821 caggccctct cttgctgtac tccccaccaa gtgctatata gagatgaaat tgaaatgaag
2881 aacttactta attgttatgc cttgaaccat tttgatactt tttattagtc ctaagtaggt
2941 attttggaaa ttgttgctta atttttaatg tttaacgcag ttgcaatata tttttggagt
3001 catattttgc tcaagaagtt tattatatac aattatacta tatatataca ccatttagca
3061 tgtactgagt ttgttggtta tttggttatc ttatacttgt gcgtggatca caaaacattc
3121 atataaggcc atgcaatata ttgttttagg ttagggtgtt gtctagatta tgctgaaagt
3181 gtaatatata tttaatttta aacaaagaac tatttttata tgaatatgta taatatacaa
3241 actatttc

11. SEQ ID NO: 11 Accession No. NM_176065 Drosophila melanogaster Hormone receptor-like in 38 CG1864-PC

MDEDCFPPLSGGWSASPPAPSQLQQLHTLQSQAQMSHPNSSNNS
SNNAGNSHNNSGGYNYHGHFNAINASANLSPSSSASSLYEYNGVSAADNFYGQQQQQQ
QQSYQQHNYNSHNGERYSLPTFPTISELAAATAAVEAAAAATVSSPSVGGPPPVRRAS
LPVQRTVSPAGSTAQSPKLAKITLNQRHSHAHAHALQLNSAPNSAASSPASADLQAGR
LLQAPSQLCAVCGDTAACQHYGVRTCEGCKGFFKRTVQKGSKYVCLADKNCPVDKRRR
NRCQFCRFQKCLVVGMVKEVVRTDSLKGRRGRLPSKPKSPQESPPSPPISLITALVRS
HVDTTPDPSCLDYSHYEEQSMSEADKVQQFYQLLTSSVDVIKQFAEKIPGYFDLLPED
.QELLFQSASLELFVLRLAYRARIDDTKLIFCNGTVLHRTQCLRSFGEWLNDIMEFSRS
LHNLEIDISAFACLCALTLITERHGLREPKKVEQLQMKIIGSLRDHVTYNAEAQKKQH

YFSRLLGKLPELRSLSVQGLQRIFYLKLEDLVPAPALIENMFVTTLPF

12. SEQ ID NO: 12 Accession No. NM_176065 Drosophila melanogaster Hormone receptor-like in 38 CG1864-PC

1 ctcgcccatt ggagggcccc tgtcctgtgg cagcagcttg cccagcttcc aggagaccta
61 ctccttgaag tacaacagca gcagcggtag cagcccccag caggcgtcct cctcctccac
121 cgccgccccc acgcccactg accaggtgct gaccctcaag atggacgagg actgcttccc
181 gcctctgtcc ggcggctgga gtgccagtcc gcccgccccc tcccagctcc agcagctgca
241 caccctgcag tctcaggccc agatgtcgca tcccaacagc agcaacaaca gcagcaacaa
301 cgcgggcaac agccacaaca acagtggggg ctacaactac cacggccact tcaatgccat
361 caatgccagc gccaatctgt cgcccagctc ctcggccagt tcctctacg aatataatgg
421 tgtttccgca gcggacaact tctacggaca acagcagcag cagcaacagc aaagctatca
481 gcaacataac tacaactcgc acaatggcga gcgttactcg ctgcccacgt ttcccacgat
541 ttcggagctg gctgcggcca ctgctgctgt cgaagctgcg gcggcggcca cagtctcctc
601 cccttcggtg ggcggtccgc cgccagtacg ccgagcatcg ctgccggttc agcgaaccgt
661 ttcgccagcc ggctccacgg cgcagagccc caagctggcc aagatcacac tgaaccagcg

721 gcactcccat gcccatgccc atgccctaca gctcaactcg gcacccaatt cggcggcaag
781 ttcgccagcg agtgcggatc tgcaggcggg ccgtttgctc caggctccgt cgcagctgtg
841 tgccgtttgt ggcgacaccg ccgcctgcca gcattatgga gtgcgaacct gcgagggatg
901 caagggattc ttcaagcgga ccgtgcagaa gggctccaag tatgtctgcc tagcggacaa
961 gaattgcccg gtggacaaga ggcgccgcaa ccgttgccag ttctgccggt tccagaagtg
1021 cctggtcgta ggcatggtca aggaagtggt gcgcacggac tcgttgaagg gtcgccgcgg
1081 gagactgccc tcaaaaccga aatcgcccca ggagtcgcca ccatcaccac ccatctcgtt
1141 gatcacggcc ctggttcgca gccatgtcga cacgactccg gatccctcgt gcctggacta
1201 cagccactat gaggagcagt cgatgagcga ggcagataag gtgcaacagt tttaccagct
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1321 tctcctgccg gaggatcagg agctgctctt ccagagcgca tcgctggaac tgttcgtcct
1381 gcggctggcc tatcgcgcca ggatcgatga caccaagctg atcttctgca acggcacggt
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2161 caacttgttg acgttaattg ttaaattgtt taatttcaac tgtcaaaacc ggaatcaacg
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2281 cactacggac ggacaaacaa cggacagaaa cagaactcac tcttgctctc ttgccttttg
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2581 taagccgtgc ttgccctaag ctaagttaga cccgcataaa gttgatagcc caaccaagta
2641 tttcggttat ttcctagact aaggtcctaa tagttatagg ctaagactat tctgttcgat
2701 ttatcaatgc accaaacagt gcacaatgag agtataagta ccttcttgtg atgattgtgt
2761 ctgacacaga gagagttgca cacaagcaca caaactagcc gataagttac taaatacgat
2821 ctaatatcta atatatataa tataatataa tatatataag tccaagtatt cggaaatcca
2881 agaacccttg cataaccgca gttcgtacgt tccaaacgag aaaagaactt tatttaatcc
2941 tagaccactc catctaagtt ctcaaagaat cgtatgtgga tcgttggatc tgtctctcta
3001 tatatgtgtg tgtgttatct cgatagaaaa cccctctatg tgattttgtg atagattggc
3061 attgaactct atatatttat atatatatgt ctataatata tatacacgca taaatatata
3121 tttttatgtc taacttttgt atggtttatt ttatacgtac cacttttctt tgataacaaa
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3241 taccagtctt tagcgacttt ccaattaacg ttcgtattaa cgaaagacag attttctatg
3301 tgttaaattg aagacttcta taactataac taaatgcaag ctaagagcaa aaacacaaat
3361 ccacaaatcc ccaaagtgaa taacatatct cttcaagctt tcgagtgcac ggaacacgta
3421 gaaccgaaac ccaagtgtta ctaaatccat ttaataatcg gcaagccggg ggcgtcggcg
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3541 tgtagcacat gaaatgttcg acaactagat tttgtaccat cttaaagaag aacctaggcc
3601 aagctaaact aagtataaac tatgatctgc atgcggctga gctgtagcta tgagaaatat
3661 acctgcgtgg atctaagtga aatgggacac tttgaattta gatatgaaac gttctaaacg
3721 cgacgtacta actctcccaa ctgcgaactc taccaattaa gagaaattcc cagaaaatgt
3781 gtcaggattt caaagcgtcc catctcactt gaacccaccc aatcaacaaa tacaaatcct
3841 agggaagttg agaggttcag caaccataga gcaatatttc ataagaaaac gcaccttaaa
3901 ttaccgaaaa acatagatta acctgatctt gtaacgtttg ggagcgataa taagccagga
3961 ttaaacagga acagttaggt gaccaaatca gttcgaaacg agatgataga taggttcggg
4021 ttcgaaaccc taaacgcgat gccattttag ccgttacaac attggatatc aaccatgcac
4081 atgaatatga atatgaatat gaatattata gagatatatc tagctatagg aacctacttt
4141 gtacctacac gacatggaaa catcaaacct acatgcatat ttacacacat atattttgaa

4201 tagagcgacg actttacaa gttgcgtaca aagctatagc tatagcttga tatggccatc
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4441 acaattttaa atgagaactt tttaatattg ctattaaagg atatacatat acacactaac
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4561 tatacaacaa attgaaaagc aacagaagaa attggcacaa attaaattta tatagcataa
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4681 gctatttccc actacacccc caaccacaca atagataacc taagctatgt atgtacatta
4741 gctatgtata tccagcccac ttatgcgcct actactagaa atgcagaaag cagaaagaga
4801 ggtgaaacct atagacgcta tcacaaatgt ctatctgata gacatcggta ctaccaatgc
4861 tatattgcca gttgtgtaat ttactcttat ttgatcgttt catttaccag ttaagaaccc
4921 aaatcatata agtgttatga tggaagaact ataacttgca attcaattaa ctctgcaata
4981 cgataacaag caaagcgaat catttcattt cgatttaatc tttaattata tatacttaaa
5041 cgatgtaagc ccaaaacaaa cgttttttct atatctgtct tttgagcaaa ttagttatac
5101 gcaaaaccaa accgtattta cataaatgta tacaaaacaa atcgtatatt ttcattggtt
5161 tgaaataaat acataaaaca a

13. SEQ ID NO: 13 Accession No. NM_141390 Drosophila melanogaster CG10296-PA

MSNFSACAVCGDQSSGKHYGVSCCDGCSCFFKRSVRRGSSYACI
ALVGNCVVDKARRNWCPSCRFQRCLAVGMNAAAVQEERGPRNQQVALYRTGRRQAPPS
QAAPSPTPHSQALHFQILAQILVTCLRQAKANEQFALLDRCQQDAIFQVVWSEIFVLR
ASHWSLDISAMIDGCGDEQLKRLICEAHQLRADVLELNFMESLILCRKELAINAEYAV
ILGSHSKAALISLARYTLQQSNYLRFGQLLLGLRQLCLRRFDCALSCMFRSVVRDILK

TL

14. SEQ ID NO: 14 Accession No. NM_141390 Drosophila melanogaster CG10296-PA

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121 tacgcctgca tcgctctggt cgggaactgt gtggtggaca aggcgcggcg gaactggtgt
181 ccctcctgcc gcttccagcg atgcctggcc gtgggaatga acgctgctgc ggttcaggag
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301 ccatctcagg cggcgccatc cccgacgccc cactcccagg cgctgcactt ccagatcctc
361 gcccagatcc ttgtcacgtg cctgcgccag gcgaaggcca acgagcagtt cgctctgttg
421 gatcgctgcc aacaagacgc catctttcag gtggtgtgga gcgagatctt cgtcctgcga
481 gcgtcccact ggtctctgga catcagcgcc atgatcgacg gctgcggcga tgagcagctc
541 aaacggctca tttgcgaggc ccaccagcta agggccgacg tcctggaact caactttatg
601 gagtccctaa tcctgtgcag aaaagaattg gccatcaatg cggagtatgc cgttatcctg
661 ggaagccact ctaaagccgc cctgatctcc ttagcccgct acaccctgca gcaatccaac
721 tacctgcggt tcggacaact gctccttggt ctgaggcagc tgtgcctgag gcgcttcgac
781 tgcgcgcttt cttgtatgtt tcgcagcgtg gtcagggaca tcttaaaaac actttag

15. SEQ ID NO: 15 Accession No. NM_169459 Drosophila melanogaster seven up CG11502-PC

MGMRREAVQRGRVPPTQPGLAGMHGQYQIANGDPMGIAGFNGHS
YLSSYISLLLRAEPYPTSRYGQCMQPNNIMGIDNICELAARLLFSAVEWAKNIPFFPE
LQVTDQVALLRLVWSELFVLNASQCSMPLHVAPLLAAGLHASPMAADRVVAFMDHIR

IFQEQVEKLKALHVDSAEYSCLKAIVLFTTDACGLSDVTHIESLQEKSQCALEEYCRT
QYPNQPTRFGKLLLRLPSLRTVSSQVIEQLFFVRLVGKTPIETLIRDMLLSGNSFSWP

YLPSM

16. SEQ ID NO: 16 Accession No. NM_169459 Drosophila melanogaster seven up CG11502-PC

1 ctaaattgtt gttttcaaaa gaaatgaatt tctttccact cctttcagaa ttcaagaata
61 aatattgaag caatatggct tcccttgttc aaaccgatca atcgttgcaa atctttcttc
121 aagcgctcgg tgcgacgtaa tctaacttac tcttgccgcg gcagcagaaa ctgtcccata
181 gatcaacacc atcgcaatca atgtcaatat tgtcgattga agaagtgcct caaaatgggc
241 atgagacgcg aagctgttca acgtggacgc gtaccaccca ctcagcccgg tctggccggc
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421 cgatatggcc agtgcatgca acccaacaac attatgggca tcgacaacat ctgcgaactg
481 gccgcccgac tgctcttctc ggcggtcgag tgggccaaga acataccctt cttcccggag
541 ctgcaggtga ccgaccaggt ggccctgctc cggctcgtct ggtcagagct cttcgtccta
601 aacgccagcc agtgctccat gccgctccat gtggcgccac tgctggccgc cgccggactt
661 catgcctccc cgatggccgc cgatcgtgtg gtggccttca tggaccacat ccgcatcttc
721 caggagcagg tggagaagct gaaggcgctg catgtcgact ccgcggagta ctcctgcctc
781 aaggcgatcg tgctcttcac caccgatgcc tgcggcctgt ccgatgtgac gcacattgaa
841 tccctgcaag agaagtcgca gtgcgccctc gaggaatact gccggaccca gtatcccaac
901 cagcccacga gattcggcaa gctgcttctc agactgccat cgctgcgaac ggtctcctca
961 caagtcattg agcaattgtt ttttgtgcgt ctagtcggaa aaacgccaat tgaaacgctg
1021 atacgcgata tgctgctgag cggcaacagt ttctcctggc cctatctgcc ttcgatgtga
1081 cacacgatgt ggcgccaatt gacaacaact tgatcatcgg ccgcagctgt ggcggctgca
1141 acgctcaaca tcaattccgg cggaggcggc atcggcatcg gcggcggggg cagtggcagt
1201 ggcggtggcg gtagtggagg cggtggcgga gtcgttggat gtggcagcca caacgttgtc
1261 gctgccagtc atgaccagct cgccaatgtt gctgtcatgc agcaaacata cggcagcggc
1321 ggcagcagca gcagcagcat cagcggttgc cacaacggta acaacggcag cggcggcagc
1381 atttgcaatc agcagatcaa caactacggc aacaacagca acaacaatgt cggcaatcat
1441 atgagtgcag gcagtttttt cggtgggtcc aacaacagca tccacagtag tggcaatagc
1501 aataccgatt atatgaccac gccagccacc gcttatgcga caccagcgac agcagccaca
1561 tccacggtga acaccacaac gatgctgtct aattactgcg atgccgccac catgatgatg
1621 gccgctgctg cagtcaatgc aaatcaatgc ctgcagcaac atcaccagcg catgttgctc
1681 gcgggcagca gcaacagcag cagcaacaac agcagcagca acagcaacgg cgcagcagca
1741 atgccctcct catcctcgtc tggctcactg tcatctgcct catcgacccc aacagcaaca
1801 gcaactgcga ctgcaattgc aacagcaaca gcaactgcag cagcaacagc cgcgcagcaa
1861 caacagcaac aatcgccgcc aaatttaatc gatatcagcg aagttcctct cattgtggat
1921 gtcaagtagt gtaattattt atgcatctag aaatggggct ataaaccaac cttgtagata
1981 ccccgccccg cccccaccac taccacaaaa accataaaac ccccaaaaaaa aaacaattga
2041 aaaatgtaaa aaaaaaaagt tggaggatga gcgccgcgta gcttaattga ctaattttcc
2101 atttgtagct ttgttgtaa ctttgtacat aactcctcga aaaattcaag tttttctcta
2161 ggccacccca gctgtgagca aaaccaatct cagctgacat atccaagaga acttcaaaag
2221 tgaagccccc aaaaaaagta agaaggcgcc aaaaaaacgt ctttacatat gaatgtgtat
2281 aatatttaaa tggcactgag ttctacttaa ttttagacca caaacacttg aaaaaatcaa
2341 tgaaaaaata agaattgtgg aaagagaaaa atccccccta acactttcaa aagacaaaac
2401 ataaagatag ttaaaatatt tatatatgta atgtagcata tacacgtata tagtacatat
2461 atgaatatat aaacgaaact ctactcccag tggtttgcag aaatatacca aaaattttaa
2521 gctatgttta cttgatgtgt ggcaattttt atgtgtgctt tagcaatttt atttttactt
2581 taagtaaaat ttaaaattta taaacattcg attctcgact ggtttttctc ggcggatgta
2641 tctcaaagat gcttctgtat gggaaggccg aattgttgaa atacgaatgc aaaatttagc
2701 gaatttttta tttagtaacc attacgagta aaaacacaaa atgttcagtg caagtttcag
2761 ttcttaaacg atttttcgt aagcttaagc attatcttat ttatgtgtat agagtatgaa
2821 aagtttcta tattttgtaa taataaaaat ttgcgtttat aatgaa

17. SEQ ID NO: 17 Accession No. NM_079857 Drosophila melanogaster tailless CG1378-PA (tll) mRNA

MQSSEGSPDMMDQKYNSVRLSPAASSRILYHVPCKVCRDHSSGK
HYGIYACDGCAGFFKRSIRRSRQYVCKSQKQGLCVVDKTHRNQCRACRLRKCFEVGMN
KDAVQHERGPRNSTLRRHMAMYKDAMMGAGEMPQIPAEILMNTAALTGFPGVPMPMPG
LPQRAGHHPAHMAAFQPPPSAAAVLDLSVPRVPHHPVHQGHHGFFSPTAAYMNALATR
ALPPTPPLMAAEHIKETAAEHLFKNVNWIKSVRAFTELPMPDQLLLLEESWKEFFILA
MAQYLMPMNFAQLLFVYESENANREIMGMVTREVHAFQEVLNQLCHLNIDSTEYECLR
AISLFRKSPPSASSTEDLANSSILTGSGSPNSSASAESRGLLESGKVAAMHNDARSAL
HNYIQRTHPSQPMRFQTLLGVVQLMHKVSSFTIEELFFRKTIGDITIVRLISDMYSQRKI

18. SEQ ID NO: 18 Accession No. NM_079857 Drosophila melanogaster tailless CG1378-PA (tll) mRNA

1 gagtccacat cggagtaacc aaggatatat cgaatatatc acacaatccg caataccgcc
61 gtccacccaa accgttaaaa caaaaatcca aaacgactca aagatacacc agtgccaagt
121 gaaattcaat ttgtgcaagc gtttctacaa aaatcgccaa aattacgccc cacatcggta
181 tgcagtcgtc ggagggttca ccagacatga tggatcagaa atacaacagc gtgcgtcttt
241 cgccagcggc atcgagtcgc attctatacc atgtgccctg caaagtctgc agagatcaca
301 gctccggcaa gcattacggc atctacgcct gtgatggctg cgccggattc ttcaagagga
361 gcattcggag atcccggcag tatgtgtgca agtcgcagaa gcagggactc tgtgtggtgg
421 acaagacgca caggaaccaa tgtagggctt gccgactgag gaagtgcttt gaggtcggaa
481 tgaacaagga tgcagtgcag cacgagcggg gaccgcggaa ctccactctg cgtcgccaca
541 tggccatgta caaggatgcc atgatgggcg ccggcgagat gccacaaata cccgccgaaa
601 ttctgatgaa cacggctgcc ttgaccggct ttcctggagt accgatgccc atgcctggcc
661 tgccccagag ggctggtcat catcctgctc acatggctgc cttccagccg ccaccatcgg
721 ctgccgctgt cttggactta tccgtgccac gagtgcccca tcacccggtg caccaaggac
781 accacggttt cttctcgccc accgccgcct acatgaatgc cctggccact cgggccctgc
841 ccccactcc tccgctgatg gcagctgagc acatcaagga aaccgcggcg gaacacctat
901 tcaagaacgt caactggatc aagagcgtac gggccttcac cgaactgccc atgccggatc
961 agctgctcct gctggaggag tcctggaagg agttcttcat cctggccatg gcccagtacc
1021 taatgcccat gaatttcgcc cagctgctgt tcgtctacga gtccgagaat gccaaccggg
1081 agatcatggg catggtgacc cgcgaggtgc acgccttcca ggaggtgctg aaccaactgt
1141 gccatctgaa cattgacagc accgagtacg agtgtctgag ggctatttcg ctcttccgta
1201 agtcaccacc gtcggcaagt tctaccgagg atttagccaa cagctcaatc ctgacaggaa
1261 gcggcagccc gaactcctcg gcctctgctg aatccagggg tcttctggag tcgggaaaag
1321 tggcggccat gcacaacgat gccccggagtg cgctgcacaa ctacatccag aggacccatc
1381 cctcgcagcc catgcgattc cagacgctct tgggcgtggt gcagctgatg cacaaggtct
1441 caagcttcac catcgaggag ctgttcttcc gaaagaccat cggcgacatc accattgtgc
1501 gcctcatctc cgacatgtac agtcagcgca agatctgaaa agtatgtaga gcctagacta
1561 atcgccgcac tcgaagtgcc ttccaagtgc tgggaactgt gataatctcg gaagaagcgc
1621 tttggacaat actcgatcag tgaaatcaac gatttctcat atccaggagt cgagccttaa
1681 aatacgtaca caacactcac cttaatacct tacctaaaca gaactcgaag taatcttagc
1741 taaagtctct cagaccatcc agatgtgttt caaattgcat tcgcaaaagt ttcaactttg
1801 cctgttaaat acgtcaatcg tagttttaaa cactttagtt ttaagcgcat attattagct
1861 ttaggatttg gaaaaataat tattc

19. SEQ ID NO:19 Accession No. NM_057792 Drosophila melanogaster dissatisfaction CG9019-PA

MGTAGDRLLDIPCKVCGDRSSGKHYGIYSCDGCSGFFKRSIHRN

RIYTCKATGDLKGRCPVDKTHRNQCRACRLAKCFQSAMNKDAVQHERGPRKPKLHPQL
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LVTNVSASFNYTQHISTHPPAPAAPPSGFHLTASGAQQGPAPPAGHLHHGGAGHQHAT
AFHHPGHGHALPAPHGGVVSNPGGNSSAISGSGPGSTLPFPSHLLHHNLIAEAASKLP
GITATAVAAVVSSTSTPYASAAQTSSPSSNNHNYSSPSPSNSIQSISSIGSRSGGGEE
GLSLGSESPRVNVETETPSPSNSPPLSAGSISPAPTLTTSSGSPQHRQMSRHSLSEAT
TPPSHASLMICASNNNNNNNNNNNNGEHKQSSYTSGSPTPTTPTPPPPRSGVGSTCNT
ASSSSGFLELLLSPDKCQELIQYQVQHNTLLFPQQLLDSRLLSWEMLQETTARLLFMA
VRWVKCLMPFQTLSKNDQHLLLQESWKELFLLNLAQWTIPLDLTPILESPLIRERVLQ
DEATQTEMKTIQEILCRFRQITPDGSEVGCMKAIALFAPETAGLCDVQPVEMLQDQAQ
CILSDHVRLRYPRQATRFGRLLLLLPSLRTIRAATIEALFFKETIGNVPIARLLRDMY

TMEPAQVDK

20. SEQ ID NO:20 Accession No. NM_057792 Drosophila melanogaster dissatisfaction CG9019-PA

1 gtcagcccag gcgatccgca tttgcgtccg cagcaggttt ccgatttcag aactctgatt
61 ccagcggcag cgaatcgcgt cggcatctga acatttgaaa ataatctaaa attgcaagtg
121 actttgtgca ccggttacac taaaattgtt aacaaatcgc catatattct gaatttaaat
181 ttaaagtgcg cagtgcggaa tataaatcag agcaaactgg atacgttagg gttcaaatac
241 ttccatcaac ggaaaatggg cacagcgggc gatcgcctgt tggacattcc ctgcaaggtg
301 tgtggcgatc gcagctccgg caagcactat ggaatctaca gctgcgatgg ctgctccggt
361 tttttcaagc ggagcattca tcgcaatcgg atttacacct gtaaggccac cggcgatctc
421 aagggtcgct gtccggtgga caagacccat cggaatcagt gtcgcgcctg tcgcctggcc
481 aagtgcttcc agtcggccat gaacaaggat gctgtgcagc acgagcgcgg tcctaggaaa
541 cccaagttgc acccgcaact gcatcatcat catcatcatg ctgctgccgc cgccgctgca
601 gcgcatcatg cagcagccgc ccatcaccat caccatcatc accaccacgc ccacgcagcg
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901 cacgccacgg ccttccacca tccgggacat ggacacgcgc tgcctgcccc acatggcggc
961 gtcgtcagca atcccggcgg caactcgagc gcaatctccg gcagcggtcc cggctccacg
1021 ctgcccttcc cctcgcacct gctgcaccac aatctgatag cggaggcggc cagcaagctg
1081 ccgggcatca ctgccacagc cgttgcggcg gtggtgtcct ccactagcac gccctacgcc
1141 tcggcggccc agacgtcgtc gcctagtagc aacaaccaca actactcctc gccctcgccc
1201 agcaactcca tccagtccat ctcgagcatt ggatcgcgca gcggtggtgg cgaggagggc
1261 ctcagcctgg gcagcgagag tccgcgcgtc aatgtggaaa cggagacacc ttcgccatcg
1321 aactcgccgc cccttagtgc tggtagcatt tcgccagcgc ccacgttgac cacctcgtcg
1381 ggatcgccgc agcaccgcca gatgtcgcgg cacagcctca gtgaggcaac cacgccgccc
1441 agccacgcct ctctcatgat ttgcgccagc aacaataaca ataacaacaa taataataac
1501 aataatggag agcacaagca gtcgagctac acatccggat caccgacacc cacaacgccc
1561 acgccgccac cgccgcgttc tggtgtaggt tccacctgca acacggccag cagctccagc
1621 ggcttcctgg agctgctgct cagtccggac aagtgccagg agctcatcca gtaccaggtg
1681 cagcacaaca cgctgctctt cccgcaacag ctgttggact cgcggctgct ctcctgggag
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1801 atgcccttcc agacgctctc caagaacgac cagcatttgc tgctccagga atcctggaag
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1981 acgatccagg agatcctctg ccgcttccgc cagatcacac ccgacggcag cgaggtgggc
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2101 gtggagatgt tgcaggatca ggcgcagtgc atcctctccg accatgtgcg actgcgctac
2161 cctcgccaag caacccgctt cggcaggctg ctgctcctgc tgccctcgct gcgcaccatc
2221 cgggcggcca ccatcgaggc gctgttcttc aaggagacca tcggcaatgt gcccattgct
2281 cgactgctgc gcgacatgta caccatggaa ccggcacagg tggacaagtg aaccggccac

2341 gcatgacagt cgaaatgaaa tcaaaatcga ttccctagca cctaagcgcc acccatcggt
2401 cgtcgtcata tgcgaactta tttgtattcc aatgcgaccc gaatcctatt cagattcact
2461 gcggcaggag gcggtccaaa tgtggggcgg aagctgcaga tgctatggtt cgcaggacgc
2521 catgtaatgg aggcgtatgt actaaccgcg ctcctccatt ggcgatgcag tccgcgatga
2581 tggcgcactc ccacacccac acccgtaccc acaccttgat ttatcgccgg caatgcgtcg
2641 gagtctcctt actttcgctt cgttttctaa catttgtatc cttattttat ttcatctttt
2701 tccacggatt tttcgttttg actgcctggg cggcactctt tatttatctt tcattcgacg
2761 ttttgtcgtc gcttttctaa aaattcccca tgttatttca acctggcaag gacctcgcag
2821 tcccattccc gcgcccttac ttacaaatca cttcccatcc cacatccagc aattccgtgg
2881 tttgaattct ttcgtgcatt gactacgaaa taccctttaa tcagacaaat aaagaatatt
2941 agttgtaatt cttttttctg caatccagct ctaaaacggg tttcttaatc gaaatcgata
3001 aatgtaaaaa ttatacatat cctttaccaa cattgtttgc cta

21. SEQ ID NO: 21 NM_166092 Drosophila melanogaster CG16801-PA

MATGRSLLFRVPWYVCLCVCAESAEPGVYWRLRLRLGLPTLAGP
HTNTLTLTARTSSCRSIKKERIKASQQANAPPELPLKVSVDVNIIIAAHSQRRRIGLV
RFHQRESEDRPLAVASPRLQINMEPTAMNPKKLHSPQRHCYTPPPAPMHGQAPPPTST
GVAPPTQPPPPHPAAPNVPNGRLLSWNHSAAAAAAAAAAQAAANSMNHSSAAEGSSMT
RIKGQNLGLICVVCGDTSSGKHYGILACNGCSGFFKRSVRRKLIYRCQAGTGRCVVDK
AHRNQCQACRLKKCLQMGMNKDDDSIDVTNDNEEPHAVSRSDSSFIMPQFMSPNLYTH
QHETVYETSARLLFMAVKWAKNLPSFARLSFRDQVILLEESWSELFLLNAIQWCIPLD
PTGCALFSVAEHCNNLENNANGDTCITKEELAADVRTLHEIFCKYKAVLVDPAEFACL
KAIVLFRPETRGLKDPAQIENLQDQAHHTKTQFTAQIARFGRLLLMLPLLRMISSHKI

ESIYFQRTIGNTPMEKVLCDMYKN

22. SEQ ID NO: 22 NM_166092 Drosophila melanogaster CG16801-PA

1 atggcgaccg ggcgttctct gctctttcga gtgccttggt atgtgtgctt gtgtgtgtgc
61 gcagagagcg cagagccggg tgtttattgg agattgcgat tgcggcttgg cttacccaca
121 ctcgcagggc cgcacaccaa cactaacact ctaacagcga ggacaagctc ctgccgcagc
181 atcaagaagg aacgaatcaa agcaagccaa caagcaaatg cgccaccaga gttgccacta
241 aaagtctccg ttgacgttaa catcatcatc gcggcacact cgcagcgccg tcggatcgga
301 ttggttcggt ttcatcagcg ggaatcagag gaccgtccac ttgccgtcgc ctctccacga
361 ttgcaaatta atatggagcc tactgcgatg aacccgaaaa aactccacag tccgcagcgg
421 cattgctaca ctccgccgcc ggcgccgatg cacggacagg cgcctccacc tacatcaacg
481 ggcgtggccc cgcccacaca gccaccgccc cctcatcccg ccgcccccaa cgtgcccaat
541 ggtcgattgc tgagctggaa tcacagtgcc gctgcagctg ctgcggcggc ggcagcccaa
601 gcggcagcca actccatgaa ccactcgtcg gcggcggagg gttcatcgat gacccggatt
661 aagggtcaga acctgggcct catctgcgtg gtgtgcggcg acaccagctc gggaaagcac
721 tacggaatcc tagcctgcaa tggctgctcc ggattcttca aacgcagcgt gcggcggaaa
781 ctcatttatc gctgccaggc gggaacggga cgctgtgtgg tggacaaagc tcatcggaat
841 caatgccagg cctgcaggct caagaagtgc cttcaaatgg gaatgaacaa ggacgacgac
901 tccatagatg taaccaacga caacgaggag ccgcatgcag tcagcagatc ggattcgagt
961 ttcattatgc cgcagttcat gtcgcccaat ctgtacaccc atcaacacga aacagtttac
1021 gagacaagtg cccggctgct cttcatggcc gtcaagtggg ccaagaacct gcccagcttt
1081 gcaagacttt cctttcggga tcaggtaatt ttgctggagg agtcctggtc ggagctgttc
1141 ctgctgaacg caatccaatg gtgcattccc ctggatccca ccggctgcgc cctcttctcg
1201 gtggcggagc actgcaataa tctagagaac aatgccaatg gcgacacttg cataacaaag
1261 gaggagctgg cggcggatgt gcgaacgctc cacgagatct tctgcaaata caaggcggtg
1321 ctggtggacc ccgctgaatt cgcgtgcctc aaggcgatag ttctcttccg gccggaaacg
1381 cgcggactta aagatccggc gcagatagag aatcttcagg atcaggcgca ccacacaaag
1441 acgcagttca ccgcccagat agccagattc ggacgactcc ttctcatgct gccgttgctg
1501 cgcatgatca gctcccacaa gattgagtcc atctatttc agcgcactat tgggaacacg
1561 cccatggaaa aggtgctctg tgacatgtat aagaactag

23. SEQ ID NO: 23 Accession No. NM_168258 Drosophila melanogaster estrogen-related receptor CG7404-PA (ERR)

MSDGVSILHIKQEVDTPSASCFSPSSKSTATQSGTNGLKSSPSV
SPERQLCSSTTSLSCDLHNVSLSNDGDSLKGSGTSGGNGGGGGGTSGGNATNASAGA
GSGSVRDELRRLCLVCGDVASGFHYGVASCEACKAFFKRTIQGNIEYTCPANNECEIN
KRRRKACQACRFQKCLLMGMLKEGVRLDRVRGGRQKYRRNPVSNSYQTMQLLYQSNTT
SLCDVKILEVLNSYEPDALSVQTPPPQVHTTSITNDEASSSSGSIKLESSVVTPNGTC
IFQNNNNNDPNEILSVLSDIYDKELVSVIGWAKQIPGFIDLPLNDQMKLLQVSWAEIL
TLQLTFRSLPFNGKLCFATDVWMDEHLAKECGYTEFYYHCVQIAQRMERISPRREEYY
LLKALLLANCDILLDDQSSLRAFRDTILNSLNDVVYLLRHSSAVSHQQQLLLLLPSLR

QADDILRRFWRGIARDEVITMKKLFLEMLEPLAR

24. SEQ ID NO: 24 Accession No. NM_168258 Drosophila melanogaster estrogen-related receptor CG7404-PA (ERR)

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   1 ccctggtcag gtctggttca ccaaaaaaga aaataaaatt acatttcaat ctttccaata
  61 tgcaaatatc tgcacgaaaa ccagcgagaa cagcatgctc acaataaaga gcccccaaac
 121 aatgtgactc gtatccgcgc agagtgacgt ttcgtgcctt gcccgagtgc caaatccaaa
 181 tcccaatcca ggcgcacaaa atcgatgcag atgctgtctg cattctcata gaaagtgcaa
 241 ctgaataacc gatggtcgcc aaaagccacg atgtccagta ataatgacca gtgaataaac
 301 aattatgact cgagcatcga aaaatgctga ggaacgaata cataagcaat aacaagaagg
 361 tgctcaactc ggaccaaaac aagtactaca tgctaacggt cgaggaggcc gatatgtatt
 421 gacgttgtta cagtggagct gattacacaa aagatcctca gaacgatttt atccaaggca
 481 cgaacatgtc cgacggcgtc agcatcttgc acatcaaaca ggaggtggac actccatcgg
 541 cgtcctgctt tagtcccagc tccaagtcaa cggccacgca gagtggcaca aacggcctga
 601 aatcctcgcc ctcggtttcg ccggaaaggc agctctgcag ctcgacgacc tctctatcct
 661 gcgatttgca caatgtatcc ttaagcaatg atggcgatag tctgaaagga agtggtacaa
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 841 gcgatgtggc cagtggattc cactatggtg tggcgagttg tgaggcttgc aaagcgttct
 901 ttaaacgcac catccaaggc aacatcgagt acacgtgtcc ggcgaacaac gagtgtgaga
 961 ttaacaagcg gagacgcaag gcctgccaag cgtgtcgctt ccagaaatgt ctactaatgg
1021 gcatgctcaa ggagggtgtg cgcttggatc gagttcgtgg aggacggcag aagtaccgaa
1081 ggaatcctgt atcaaactct taccagacta tgcagctgct ataccaatcc aacaccacct
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1321 aaaacaacaa caacaatgat cccaatgaga tactaagcgt ccttagtgat atttacgaca
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1441 ttaacgacca gatgaagctt ctccaggtgt cgtgggcaga gatcctgacg ctccagctga
1501 ccttccggtc cctaccgttc aatggcaagt tatgcttcgc cacggatgtc tggatggatg
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1621 agcgcatgga aagaatatcg ccacgaaggg aggagtacta cttgctaaag gcgctcctgc
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1801 agcaacaatt gctgcttttg ctgccttcgc tgcggcaggc ggatgatatc ctgcgaagat
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1921 tcgagccgct ggccaggtga aaaggattat gcgggcgccc aaactagttg atctagctga
1981 taagcaaagg tgcaaatata gtcttaggta tatatggatg tatactagag tagattaagc
2041 gtaggataag ccatgtatat aaatagtaaa atacttgtcg ggtaagatta gttcgcagaa
2101 aaaatctctt ttaatggact accaactaca gcaactggaa aaccctactt atcttctaga
2161 atcggggtgt gcttacactg gttaaaggcg catataggtg ttatgtgtct aaagttgtga
```

2221 gtcacagatc ttcaataatt tgttcaattc tcactggttc tgatatatgt atatgccgca
2281 accttctgat gtaacgtatg aatttgtggg cactttaaa atacgatagt ggttctacaa
2341 tacaatggat tatactgttt ctaagtgtca tgtaacccag tgattctgtg tctatgtggt
2401 acacatgcgg tcaaaagaat agcaatgtcg tccgtgaata ataaaccgtt tgtaactgtt
2461 gtttccatac tccctaagtt ctgtattctt tggggatttt cttttcctaa acaaattcaa
2521 attagtttt

25. SEQ ID NO: 25 Accession No. NM_168908 Drosophila melanogaster Hormone-receptor-like in 78 CG7199-PC

MDGVKVETFIKSEENRAMPLIGGGSASGGTPLPGGGVGMGAGAS
ATLSVELCLVCGDRASGRHYGAISCEGCKGFFKRSIRKQLGYQCRGAMNCEVTKHHRN
RCQFCRLQKCLASGMRSDSVQHERKPIVDRKEGIIAAAGSSSTSGGGNGSSTYLSGKS
GYQQGRGKGHSVKAESAATPPVHSAPATAFNLNENIFPMGLNFAELTQTLMFATQQQQ
QQQQQHQQSGSYSPDIPKADPEDDEDDSMDNSSTLCLQLLANSASNNNSQHLNFNAGE
VPTALPTTSTMGLIQSSLDMRVIHKGLQILQPIQNQLERNGNLSVKPECDSEAEDSGT
EDAVDAELEHMELDFECGGNRSGGSDFAINEAVFEQDLLTDVQCAFHVQPPTLVHSYL
NIHYVCETGSRIIFLTIHTLRKVPVFEQLEAHTQVKLLRGVWPALMAIALAQCQGQLS
VPTIIGQFIQSTRQLADIDKIEPLKISKMANLTRTLHDFVQELQSLDVTDMEFGLLRL
ILLFNPTLLQQRKERSLRGYVRRVQLYALSSLRRQGGIGGGEERFNVLVARLLPLSSL

DAEAMEELFFANLVGQMQMDALIPFILMTSNTSGL

26. SEQ ID NO: 26 Accession No. NM_168908 Drosophila melanogaster Hormone-receptor-like in 78 CG7199-PC

1 attggaacaa ggagatttta ttgcgttaga aaaggttcaa aataggcaca aagtgcctga
61 aaatatcgta actgaccgga agtaacataa ctttaaccaa gtgcctcgaa aaatagatgt
121 ttttaaaagc tcaagaatgg tgataacaga cgtccaataa gaattttcaa agagccaaat
181 gtttgggttt cagttattta tacagccgac gactattttt tagccgcctg ctgtggcgac
241 aatggacggc gttaaggttg agacgttcat caaaagcgaa gaaaaccgag cgatgccctt
301 gatcggagga ggcagtgcct caggcggcac tcctctgcca ggaggcggcg tgggaatggg
361 agccggagca tccgcaacgt tgagcgtgga gctgtgtttg gtgtgcgggg accgcgcctc
421 cgggcggcac tacggagcca taagctgcga aggctgcaag ggattcttca agcgctcgat
481 ccggaagcag ctgggctacc agtgtcgcgg ggctatgaac tgcgaggtca ccaagcacca
541 caggaatcgg tgccagttct gtcgactaca gaagtgcctg gccagcggca tgcgaagtga
601 ttctgtgcag cacgagagga aaccgattgt ggacaggaag gaggggatca tcgctgctgc
661 cggtagctca tccacttctg gcggcggtaa tggctcgtcc acctacctat ccggcaagtc
721 cggctatcag caggggcgtg gcaaggggca cagtgtaaag gccgaatccg cggccacgcc
781 tccagtgcac agcgcgccag caacggcctt caatttgaat gagaatatat tcccgatggg
841 tttgaatttc gcagaactaa cgcagacatt gatgttcgct acccaacagc agcagcaaca
901 acagcaacag catcaacaga gtggtagcta ttcgccagat attccgaagg cagatcccga
961 ggatgacgag gacgactcaa tggacaacag cagcacgctg tgcttgcagt tgctcgccaa
1021 cagcgccagc aacaacaact cgcagcacct gaactttaat gctggggaag tacccaccgc
1081 tctgcctacc acctcgacaa tggggcttat tcagagttcg ctggacatgc gggtcatcca
1141 caagggactg cagatcctgc agcccatcca aaaccaactg gagcgaaatg gtaatctgag
1201 tgtgaagccc gagtgcgatt cagaggcgga ggacagtggc accgaggatg ccgtagacgc
1261 ggagctggag cacatggaac tagactttga gtgcggtggg aaccgaagcg gtggaagcga
1321 ttttgctatc aatgaggcgg tctttgaaca ggatcttctc accgatgtgc agtgtgcctt
1381 tcatgtgcaa ccgccgactt tggtccactc gtatttaaat attcattatg tgtgtgagac
1441 gggctcgcga atcatttttc tcaccatcca tacccttcga aaggttccag ttttcgaaca
1501 attggaagcc catacacagg tgaaactcct gagaggagtg tggccagcat taatggctat
1561 agctttggcg cagtgtcagg gtcagctttc ggtgcccacc attatcgggc agtttattca
1621 aagcactcgc cagctagcgg atatcgataa gatcgaaccg ttgaagatct cgaagatggc
1681 aaatctcacc aggaccctgc acgactttgt ccaggagctc cagtcactgg atgttactga

1741 tatggagttt ggcttgctgc gtctgatctt gctcttcaat ccaacgctct tgcagcagcg
1801 caaggagcgg tcgttgcgag gctacgtccg cagagtccaa ctctacgctc tgtcaagttt
1861 gagaaggcag ggtggcatcg gcggcggcga ggagcgcttt aatgttctgg tggctcgcct
1921 tcttccgctc agcagcctgg acgcagaggc catggaggag ctgttcttcg ccaacttggt
1981 ggggcagatg cagatggatg ctcttattcc gttcatactg atgaccagca acaccagtgg
2041 actgtaggcg gaattgagaa gaacagggcg caagcagatt cgctagactg cccaaaagca
2101 agactgaaga tggaccaagt gcgggcaata catgtagcaa ctaggcaaat cccattaatt
2161 atatatttaa tatatacaat atatagttta ggatacaata ttctaacata aaaccatggg
2221 tttattgttg ttcacagata aaatggaatc gatttcccaa taaaagcgaa tatgttttta
2281 aacagaat

27. SEQ ID NO: 27 Accession No. NM_057433 Drosophila melanogaster ultraspiracle CG4380-PA (usp)

MDNCDQDASFRLSHIKEEVKPDISQLNDSNNSSFSPKAESPVPF
MQAMSMVHVLPGSNSASSNNNSAGDAQMAQAPNSAGGSAAAAVQQQYPPNHPLSGSKH
LCSICGDRASGKHYGVYSCEGCKGFFKRTVRKDLTYACRENRNCIIDKRQRNRCQYCR
YQKCLTCGMKREAVQEERQRGARNAAGRLSASGGGSSGPGSVGGSSSQGGGGGGGVSG
GMGSGNGSDDFMTNSVSRDFSIERIIEAEQRAETQCGDRALTFLRVGPYSTVQPDYKG
AVSALCQVVNKQLFQMVEYARMMPHFAQVPLDDQVILLKAAWIELLIANVAWCSIVSL
DDGGAGGGGGGLGHDGSFERRSPGLQPQQLFLNQSFSYHRNSAIKAGVSAIFDRILSE
LSVKMKRLNLDRRELSCLKAIILYNPDIRGIKSRAEIEMCREKVYACLDEHCRLEHPG
DDGRFAQLLLRLPALRSISLKCQDHLFLFRITSDRPLEELFLEQLEAPPPPGLAMKLE

28. SEQ ID NO: 28 Accession No. NM_057433 Drosophila melanogaster ultraspiracle CG4380-PA (usp)

1 aaaaatgtcg acgcgaaaaa aggtatttat tcattagtca gaaagtctgg cattctttgt
61 ttgttggtaa aaagcgcaat tgtttggagg cgagcgaata aagtgcgctg ctccatcggc
121 tcaagattat gtaaatgcag caacgacccc accaacaacg aaactgcaac ctgctccact
181 tggcccaacg gaccaatagc ggacggacgg acacggtggc gttggcaaag tgaaaccccca
241 acagagaggc gaaagcgagc caagacacac cacatacaca cgaagagaac gagcaagaag
301 aaaccggtag gcggaggagg cgctgccccc agttcctcca atatacccag caccacatca
361 caagcccagg atggacaact gcgaccagga cgccagcttt cggctgagcc acatcaagga
421 ggaggtcaag ccggacatct cgcagctgaa cgacagcaac aacagcagct ttcgcccaa
481 ggccgagagt cccgtgccct tcatgcaggc catgtccatg gtccacgtgc tgcccggctc
541 caactccgcc agctccaaca acaacagcgc tggagatgcc caaatggcgc aggcgcccaa
601 ttcggctgga ggctctgccg ccgctgcagt ccagcagcag tatccgccta accatccgct
661 gagcggcagc aagcacctct gctctatttg cggggatcgg gccagtggca agcactacgg
721 cgtgtacagc tgtgagggct gcaagggctt ctttaaacgc acagtgcgca aggatctcac
781 atacgcttgc agggagaacc gcaactgcat catagacaag cggcagagga accgctgcca
841 gtactgccgc taccagaagt gcctaacctg cggcatgaag cgcgaagcgg tccaggagga
901 gcgtcaacgc ggcgcccgca atgcggcggg taggctcagc gccagcggag gcggcagtag
961 cggtccaggt tcggtaggcg gatccagctc tcaaggcgga ggaggaggag gcggcgtttc
1021 tggcggaatg ggcagcggca acggttctga tgacttcatg accaatagcg tgtccaggga
1081 tttctcgatc gagcgcatca tagaggccga gcagcgagcg gagacccaat gcggcgatcg
1141 tgcactgacg ttcctgcgcg ttggtcccta ttccacagtc cagccggact acaagggtgc
1201 cgtgtcggcc ctgtgccaag tggtcaacaa acagctcttc cagatggtcg aatacgcgcg
1261 catgatgccg cactttgccc aggtgccgct ggacgaccag gtgattctgc tgaaagccgc
1321 ttggatcgag ctgctcattg cgaacgtggc ctggtgcagc atcgtttcgc tggatgacgg
1381 cggtgccggc ggcgggggcg gtggactagg ccacgatggc tcctttgagc gacgatcacc
1441 gggccttcag ccccagcagc tgttcctcaa ccagagcttc tcgtaccatc gcaacagtgc
1501 gatcaaagcc ggtgtgtcag ccatcttcga ccgcatattg tcggagctga gtgtaaagat
1561 gaagcggctg aatctcgacc gacgcgagct gtcctgcttg aaggccatca tactgtacaa
1621 cccggacata cgcgggatca agagccgggc ggagatcgag atgtgccgcg agaaggtgta

1681 cgcttgcctg gacgagcact gccgcctgga acatccgggc gacgatggac gctttgcgca
1741 actgctgctg cgtctgcccg ctttgcgatc gatcagcctg aagtgccagg atcacctgtt
1801 cctcttccgc attaccagcg accggccgct ggaggagctc tttctcgagc agctggaggc
1861 gccgccgcca cccggcctgg cgatgaaact ggagtagggt cccgactcta aagtctcccc
1921 cgttctccat ccgaaaaatg tttcattgtg attgcgtttg tttgcatttc tcctctctat
1981 cccttatacc ctacaaaagc cccctaatat tacgcaaaat gtgtatgtaa ttgtttattt
2041 ttttttatt acctaatatt attattatta ttgatataga aaatgttttc cttaagatga
2101 agattagcct cctcgacgtt tatgtcccag taaacgaaaa acaaacaaaa tccaaaactt
2161 gaaaagaaca caaaacacga acgagaaaat gcacacaagc aaagtaaaag taaaagttaa
2221 actaaagcta aacgagtaaa gatattaaaa taacggttaa aattaatgca tagttatgat
2281 ctacagacgt atgtaaacat acaaattcag cataaatata tatgtcagca ggcgcatatc
2341 tgcggtgctg gccccgttct aaatcaattg taattacttt ttaacataaa tttacccaaa
2401 acgttatcaa ttagatgcga gatacaaaaa tcaccgacga aaaccaacaa aatatatcta
2461 tgtataaaaa atataaactg cataacaa

29. SEQ ID NO: 29 Accession No. NM_168757 Drosophila melanogaster Ecdysone-induced protein 75B CG8127-PD

MGEELPILKGILKGNVNYHNAPVRFGRVPKREKARILAAMQQST
QNRGQQRALATELDDQPRLLAAVLRAHLETCEFTKEKVSAMRQRARDCPSYSMPTLLA
CPLNPAPELQSEQEFSQRFAHVIRGVIDFAGMIPGFQLLTQDDKFTLLKAGLFDALFV
RLICMFDSSINSIICLNGQVMRRDAIQNGANARFLVDSTFNFAERMNSMNLTDAEIGL
FCAIVLITPDRPGLRNLELIEKMYSRLKGCLQYIVAQNRPDQPEFLAKLLETMPDLRT
LSTLHTEKLVVFRTEHKELLRQQMWSMEDGNNSDGQQNKSPSGSWADAMDVEAAKSPL
GSVSSTESADLDYGSPSSSQPQGVSLPSPPQQQPSALASSAPLLAATLSGGCPLRNRA
NSGSSGDSGAAEMDIVGSHAHLTQNGLTITPIVRHQQQQQQQQQIGILNNAHSRNLNG
GHAMCQQQQQHPQLHHHLTAGAARYRKLDSPTDSGIESGNEKNECKAVSSGGSSSCSS
PRSSVDDALDCSDAAANHNQVVQHPQLSVVSVSPVRSPQPSTSSHLKRQIVEDMPVLK
RVLQAPPLYDTNSLMDEAYKPHKKFRALRHREFETAEADASSSTSGSNSLSAGSPRQS
PVPNSVATPPPSAASAAAGNPAQSQLHMHLTRSSPKASMASSHSVLAKSLMAEPRMTP
EQMKRSDIIQNYLKRENSTAASSTTNGVGNRSPSSSSTPPPSAVQNQQRWGSSSVITT
TCQQRQQSVSPHSNGSSSSSSSSSSSSSSSSSTSSNCSSSSASSCQYFQSPHSTSNGT
SAPASSSSGSNSATPLLELQVDIADSAQPLNLSKKSPTPPPSKLHALVAAANAVQRYP

TLSADVTVTASNGGPPSAAASPAPSSSPPASVGSPNPGLSAAVHKVMLEA

30. SEQ ID NO: 30 Accession No. NM_168757 Drosophila melanogaster Ecdysone-induced protein 75B CG8127-PD

1 agtcaccgtc gcagtcgcag cagttgaggt tcgctctcct cgatttcggg caaatccgat
61 accatatagc acagcgtacc gcactctggg tatattcgta acgcgctttg gcttttacag
121 ttagtcgcgt tcgagacctt gtcgagtttt gtcatgttag ccagcgatcc gcgggatccg
181 aaataagcca agaatcacaa cgcgagtgcg gcagttgcca gcagtaacta caccaatatt
241 tatattaatt aaaataaatt aaatgaaaca acatgctgat taatgccaat gaatgttaaa
301 tgcaattgtt aatgtgaaga aaagtcgacc aagtctcccc aaaacaacac ttattcaaca
361 tccactacac actcgccttt ctggattacg cgcccaaaaa aaaacaaaaa ttaaaaatta
421 aaccaaacca acaactaatt tatttgctaa atattccaaa aattcaatca atgtgaaaag
481 caagcaaaca aagttcctct cacaacaaaa cagcagttaa ttaaaatatc taaccgagat
541 aaagtgcaaa gaagataaca agtttctcaa gcaaacatcc atatgtacct gagtaccaac
601 caaaaagctg tgtgtgtgcc aaaaaccgaa gaggaattat ccaaaaatat ttaatgagca
661 agctcaactg agtggttgat gtgcccccca agggaaaagt gaccaagtca agatattttg
721 tcaaatcgaa cacagaaaac acaaaaatgg gcgaagaact cccgatattg aagggcatac
781 ttaaaggcaa cgtcaactat cacaatgcgc ctgtgcgttt tggacgcgtg ccgaagcgcg
841 aaaaggcgcg tatcctggcg gccatgcaac agagcaccca gaatcgcggc cagcagcgag
901 ccctcgccac cgagctggat gaccagccac gcctcctcgc cgccgtgctg cgcgcccacc

961 tcgagacctg tgagttcacc aaggagaagg tctcggcgat gcggcagcgg gcgcgggatt
1021 gcccctccta ctccatgccc acacttctgg cctgtccgct gaaccccgcc cctgaactgc
1081 aatcggagca ggagttctcg cagcgtttcg cccacgtaat tcgcggcgtg atcgactttg
1141 ccggcatgat tcccggcttc cagctgctca cccaggacga taagttcacg ctcctgaagg
1201 cgggactctt cgacgccctg tttgtgcgcc tgatctgcat gtttgactcg tcgataaact
1261 caatcatctg tctaaatggc caggtgatgc gacgggatgc gatccagaac ggagccaatg
1321 cccgcttcct ggtggactcc accttcaatt tcgcggagcg catgaactcg atgaacctga
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1441 tgcgcaacct ggagctgatc gagaagatgt actcgcgact caagggctgc ctgcagtaca
1501 ttgtcgccca gaataggccc gatcagcccg agttcctggc caagttgctg gagacgatgc
1561 ccgatctgcg caccctgagc accctgcaca ccgagaaact ggtagttttc cgcaccgagc
1621 acaaggagct gctgcgccag cagatgtggt ccatggagga cggcaacaac agcgatggcc
1681 agcagaacaa gtcgccctcg ggcagctggg cggatgccat ggacgtggag gcggccaaga
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1801 gttcgcagcc acagggcgtg tctctgccct cgccgcctca gcaacagccc tcggctctgg
1861 ccagctcggc tcctctgctg gcggccaccc tctccggagg atgtcccctg cgcaaccggg
1921 ccaattccgg ctccagcggt gactccggag cagctgagat ggatatcgtt ggctcgcacg
1981 cacatctcac ccagaacggg ctgacaatca cgccgattgt gcgacaccag cagcagcaac
2041 aacagcagca gcagatcgga atactcaata atgcgcattc ccgcaacttg aatgggggac
2101 acgcgatgtg ccagcaacag cagcagcacc cacaactgca ccaccacttg acagccggag
2161 ctgcccgcta cagaaagcta gattcgccca cggattcggg cattgagtcg ggcaacgaga
2221 agaacgagtg caaggcggtg agttcggggg gaagttcctc gtgctccagt ccgcgttcca
2281 gtgtggatga tgcgctggac tgcagcgatg ccgccgccaa tcacaatcag gtggtgcagc
2341 atccgcagct gagtgtggtg tccgtgtcac cagttcgctc gccccagccc tccaccagca
2401 gccatctgaa gcgacagatt gtggaggata tgcccgtgct gaagcgcgtg ctgcaggctc
2461 cccctctgta cgataccaac tcgctgatgg acgaggccta caagccgcac aagaaattcc
2521 gggccctgcg gcatcgcgag ttcgagaccg ccgaggcgga tgccagcagt tccacttccg
2581 gctcgaacag cctgagtgcc ggcagtccgc gacagagtcc agtcccgaac agtgtggcca
2641 cgcccccgcc atcggcggcc agcgccgccg caggtaatcc cgcccagagc cagctgcaca
2701 tgcacctgac ccgcagcagc cccaaggcct cgatggccag ctcgcactcg gtgctggcca
2761 agtctctcat ggccgagccg cgcatgacgc ccgagcagat gaagcgcagc gatattatcc
2821 aaaactactt gaagcgcgag aacagcacag cagccagcag caccaccaat ggcgtgggca
2881 accgcagtcc cagcagcagc tccacaccgc cgccatcggc ggtccagaat cagcagcgtt
2941 ggggcagcag ctcggtgatc accaccacct gccagcagcg ccagcagtcc gtgtcgccgc
3001 acagcaacgg ttccagctcc agttcgagct ctagctccag ctccagttcg tcatcctcct
3061 ccacatcctc caactgcagc tccagctcgg ccagcagctg ccagtatttc cagtcgccgc
3121 actccaccag caacggcacc agtgcaccgg cgagctccag ttcgggatcg aacagcgcca
3181 cgcccctgct ggaactgcag gtggacattg ctgactcggc gcagcctctc aatttgtcca
3241 agaaatcgcc cacgccgccg cccagcaagc tgcacgctct ggtggccgcc gccaatgccg
3301 ttcaaaggta tcccacattg tccgccgacg tcacagtgac agcctccaat ggcggtcctc
3361 cgtcggcggc ggcgagtccg gcgcccagca gcagtccgcc ggcgagtgtg ggctccccca
3421 atccgggcct gagcgccgcc gtgcacaagg taatgctgga ggcgtaagag cgggaggagg
3481 taggtggttt tacgcggaga agtgggagag acagagactg ggagtggcag ttcagcgaag
3541 caggaagcag gatcacttgg agcggcggga gttgaattaa attattttac catttaattg
3601 agacgtgtac aaagtttgaa agcaaaacca acatgcatgc aatttaaaac taatatttaa
3661 agcaacaaca aacaaaacaa ctacaagtta ttaatttaaa aaacaaacaa acaaacaaac
3721 aacaaaaaac ccaagcttga atggtattac

31. SEQ ID NO: 31 Accession No. NM_168892 Drosophila melanogaster Ecdysone-induced protein 78C CG18023-PBEip78C)

MHPSHLQQQQQQHLLQQQQQQQHQPQLQQHHQLQQQPHVSGVRV
KTPSTPQTPQMCSIASSPSELGGCNSANNNNNNNNNNSSSGNASGGSGVSVGVVVVGGH
QQLVGGSMVGMAGMGTDAHQVGMCHDGLAGTANELTVYDVIMCVSQAHRLNCSYTEEL
TRELMRRPVTVPQNGIASTVAESLEFQKIWLWQQFSARVTPGVQRIVEFAKRVPGFCD
FTQDDQLILIKLGFFEVWLTHVARLINEATLTLDDGAYLTRQQLEILYDSDFVNALLN

FANTLNAYGLSDTEIGLFSAMVLLASDRAGLSEPKVIGRARELVAEALRVQILRSRAG
SPQALQLMPALEAKIPELRSLGAKHFSHLDWLRMNWTKLRLPPLFAEIFDIPKADDEL

32. SEQ ID NO: 32 Accession No. NM_168892 Drosophila melanogaster Ecdysone-induced protein 78C CG18023-PBEip78C)

1 aagcattaac gaaagaactg cgcacaaagt agggaggcaa taattacata tgtacatggc
61 tgggaaaggc cttaactaaa cttagcaaac taataaatag aaaaaaggaa atattggcca
121 aatattatag tattgggaat attaggttac ttgatatcaa aaattaatgt ctattttata
181 cacttattct tagacttaat gttaacttat cgtacttatt atgattggtt tttcaagatt
241 accagaactt gatagattgg tctagctttt gaaatcggat agcattttct ttaaaggact
301 ttgccatatg ctaaagccta acttcttttt tcaattcagc cacagctgac aaaagcgaag
361 aaaatttgaa agaccgtgaa tccttttgaa acgccctctc cggattcctc attaagtgca
421 aaagatataa catcgcagag atttcccata aaaatgctga tcaggcgccc tcgcaggttg
481 ccaacgtcga tttccgccag caggacgatg atgaagatga tggatgccca tctcaccgat
541 tcgatccgag caacatggat gtataccaaa tagagctgga ggaacaggca caaatccgct
601 ccaaactgct ggtcgaaacc tgtgtgaagc actcgtcttc ggagcagcag cagctccaag
661 ttaagcagga ggacctcatc aaggatttca ctcgggacga ggaggaacag ccaagcgaag
721 aggaggcgga ggaagaggac aacgaagagg acgaggaaga agaaggcgaa gaagaagagg
781 aggacgagga cgaggaagcc ctgctgccgg tagtcaattt taatgcaaat tcagacttta
841 atttgcattt ctttgacaca ccggaggact cgtccaccca aggggcctac agtgaggcca
901 atagcttgga atccgagcag gaagaggaga agcaaacaca gcagcatcag cagcagaagc
961 agcatcaccg ggatttggag gattgcctaa gtgccattga agctgatcca ttgcagttgt
1021 tgcattgcga cgacttctat agaacatcag ccctagcaga gagtgttgca gccagtctaa
1081 gcccacagca gcagcagcaa cggcagcaca cccaccagca acaacagcaa cagcagcagc
1141 agcagcaaca ccctggacag cagcaacatc agctcaactg cacgctgagc aatggtggag
1201 gtgctttgta caccatcagc agtgtgcatc agttcggtcc ggccagcaac cacaacacca
1261 gcagcagctc cccctcctcc agcgccgccc actcttcgcc ggacagcggc tgctcgtcgg
1321 cctcctcctc cggatcttcg cgatcctgcg gatcctcctc tgcatcctcc tcctcgtcag
1381 cggtcagcag caccatcagc agcggccgca gcagcaacaa cagcgtcgtc aaccccgcag
1441 caacatcttc atctgttgcg catctgaaca aagagcaaca gcagcagcca ctgccgacga
1501 cacagctgca acagcagcag cagcaccagc agcagttgca acacccgcag cagcagcaat
1561 cttttggcct agcagacagc agcagcagca acggcagcag caacaacaac aacggtgtct
1621 cctcgaaatc atttgtgccc tgcaaagtct gtggcgacaa ggcatcggga taccactatg
1681 gtgtaacctc ctgcgagggt tgcaagggat tctttcgtcg cagtatccag aagcaaatcg
1741 aatatcgctg tttgcgggac ggcaagtgcc tggtcatcag actgaaccgc aatcgctgcc
1801 agtactgccg cttcaagaaa tgcctttccg ctggcatgag ccgcgattcc gtacgttatg
1861 gtcgcgttcc caagcgttcc cgtgagctga acggagcggc cgcctcctcc gccgccgctg
1921 gagctcctgc ctccctcaat gtggatgact ctaccagcag cacactgcac ccgagtcacc
1981 tacagcagca gcagcaacag catctactac agcagcaaca gcagcagcaa catcagccac
2041 agctgcagca acaccaccaa ctgcaacagc agccgcatgt aagcggcgta cgtgtgaaga
2101 ccccgagtac tccacaaacg ccacaaatgt gttcgatcgc ctcctcgcca tcggagctgg
2161 gcggttgcaa tagtgccaat aacaataaca ataataacaa caacagtagc agcggtaatg
2221 ccagcggtgg cagcggcgtg agcgtcggcg ttgttgttgt gggcggacac cagcaactgg
2281 tgggaggcag catggtggga atggcgggca tgggcacgga tgcccaccag gtgggcatgt
2341 gtcacgacgg cttggcggga acggcaaacg agctgaccgt ctacgatgtc atcatgtgcg
2401 tgtcgcaggc gcaccgcctc aactgctcct acacggagga actgaccaga gagctcatgc
2461 gtcgtcccgt gacggtgcca caaaatggga ttgccagcac agtggccgag agtctggagt
2521 tccagaagat ctggctgtgg caacagttct cggccagggt gacgcctggc gttcagcgga
2581 ttgtggagtt tgcgaaacgc gtacctggct tctgtgattt cacccaagat gaccagctta
2641 tactaataaa gctgggcttc ttcgaggtct ggttgaccca tgtggcccgg ttgatcaatg
2701 aggcgacatt gacactggac gatggtgcct acctgacgcg ccagcagctt gagatactct
2761 acgattctga ctttgtcaac gccttgctga actttgccaa cacgctgaac gcctacgggc
2821 tgagtgacac cgaaatcgga ctcttctcgg ccatggtgct gcttgcctcg gatcgagctg
2881 gactcagcga gcccaaggtg atcggcaggg ccagggaact ggtggccgag gcgctgcgcg
2941 tacagatcct gcgttcgcgg gcaggatccc cacaggcgct gcagctgatg ccggcgctgg

3001 aagccaagat acccgagctg agatccttgg gggccaagca cttctcacac ctagactggc
3061 tacggatgaa ctggaccaag ctgcgcctgc cgcccctctt cgccgagatc ttcgacatcc
3121 cgaaggctga cgatgagctg taggatgtgg agccaacccc gcgattccag ggccgtgcaa
3181 agcaaaccgc aacaagaaca gaatattcta ccacttgtag gcttaagcaa cgtagctata
3241 gatcgaaatg ggagggccgc agatcagata cacgtctact cagcattacc ggagagatag
3301 tccactaagc ctatatgcat actactatac tagcagtgtt a

33. SEQ ID NO: 33 Accession No. NM_165465 Drosophila melanogaster Ecdysone receptor CG1765-PB (EcR)

MKRRWSNNGGFMRLPEESSSEVTSSSNGLVLPSGVNMSPSSLDS
HDYCDQDLWLCGNESGSFGGSNGHGLSQQQQSVITLAMHGCSSTLPAQTTIIPINGNA
NGNGGSTNGQYVPGATNLGALANGMLNGGFNGMQQQIQNGHGLINSTTPSTPTTPLHL
QQNLGGAGGGGIGGMGILHHANGTPNGLIGVVGGGGGVGLGVGGGGVGGLGMQHTPRS
DSVNSISSGRDDLSPSSSLNGYSANESCDAKKSKKGPAPRVQEELCLVCGDRASGYHY
NALTCEGCKGFFRRSVTKSAVYCCKFGRACEMDMYMRRKCQECRLKKCLAVGMRPECV
VPENQCAMKRREKKAQKEKDKMTTSPSSQHGGNGSLASGGGQDFVKKEILDLMTCEPP
QHATIPLLPDEILAKCQARNIPSLTYNQLAVIYKLIWYQDGYEQPSEEDLRRIMSQPD
ENESQTDVSFRHITEITILTVQLIVEFAKGLPAFTKIPQEDQITLLKACSSEVMMLRM
ARRYDHSSDSIFFANNRSYTRDSYKMAGMADNIEDLLHFCRQMFSMKVDNVEYALLTA
IVIFSDRPGLEKAQLVEAIQSYYIDTLRIYILNRHCGDSMSLVFYAKLLSILTELRTL
GNQNAEMCFSLKLKNRKLPKFLEEIWDVHAIPPSVQSHLQITQEENERLERAERMRAS
VGGAITAGIDCDSASTSAAAAAAQHQPQPQPQPQPSSLTQNDSQHQTQPQLQPQLPPQ
LQGQLQPQLQPQLQTQLQPQIQPQPQLLPVSAPVPASVTAPGSLSAVSTSSEYMGGSA
AIGPITPATTSSITAAVTASSTTSAVPMGNGVGVGVGVGGNVSMYANAQTAMALMGVA

LHSHQEQLIGGVAVKSEHSTTA

34. SEQ ID NO: 34 Accession No. NM_165465 Drosophila melanogaster Ecdysone receptor CG1765-PB (EcR)

1 tagtattttt ttggactttg ttgttaacgg ttgttcgctc gcacgtacga agcccgatcg
61 cgttcgtcaa aaaacaagat acaaaataca gcacacacaa ttgaaaacga caacctaaca
121 gtacggtttc ccaaagcacc ttacatttca aaaccgaaaa cccccaaaat gttgtaacca
181 aataatgttt aaatcacata tacacctaca tatatttatg aaaaattgtt agacaaatcc
241 caaataatac cagttccccc aacaaccgca acaaacacaa gtgcaattca tcggcaaaaa
301 ttaatataaa gtgcaaatgc attgtagctg aaactcaaac aatagtaaaa atacatacat
361 aagtggtgaa gaagcaaaag gaaatagttc ttaaaataac gcaaatcgag agcatatatt
421 catatttgta cagatattat atggcggctg catagtgcaa actgcggctg agggaataca
481 gcggtatcga aatgtaaata ggaaacaacg aagccagaac tcgaaatcaa acatcagcaa
541 cgtgacacac agacataaga cgcccgtcta gtcgtggtct gtggaacgct agctccgctt
601 tgccaggagc cggagacttt ttccgcatcc acaatattac atatgtacat atatcgaaga
661 tagtgcgcga gtgagtgagg gatttgtgcc gtggatcccg atccccttac atatatataa
721 aggtagtgaa aagattttac tcaacattcc aaatagtgct ttgtcaactg gaataccttt
781 tgttcaaata cgcagtgggc ccatggatac ttgtggatta gtagcagaac tggcgcacta
841 tatcgacgca tatgctctga ttgtttcccg cactaaatga gcagggattc gggcgaaaat
901 gtattttgaa cgcaaacaag tgcgcaaaaa atactagctc caccacgaaa ctgcacaaaa
961 caccgccaga agcgagcaga acctcgggcc gcacgaccga gcttcgtaaa gcaacagagg
1021 atcttaccag gagatagctc ttctccacat agaccaactg ccagggacaa gctccttgtc
1081 cccagccgac gctaagtgaa cggaaaacgg ccacaaaacg gcgactatcg gctgccagag
1141 gatgaagcgg cgctggtcga acaacggcgg cttcatgcgc ctaccggagg agtcgtcctc
1201 ggaggtcacg tcctcctcga acgggctcgt cctgccctcg gggtgaaca tgtcgccctc
1261 gtcgctggac tcgcacgact attgcgatca ggacctttgg ctctgcggca acgagtccgg
1321 ttcgtttggc ggctccaacg gccatggcct aagtcagcag cagcagagcg tcatcacgct
1381 ggccatgcac gggtgctcca gcactctgcc cgcgcagaca accatcattc cgatcaacgg

1441 caacgcgaat gggaatggag gctccaccaa tggccaatat gtgccgggtg ccactaatct
1501 gggagcgttg gccaacggga tgctcaatgg gggcttcaat ggaatgcagc aacagattca
1561 gaatggccac ggcctcatca actccacaac gccctcaacg ccgaccaccc cgctccacct
1621 tcagcagaac ctgggggcg cgggcggcgg cggtatcggg ggaatgggta ttcttcacca
1681 cgcgaatggc accccaaatg gccttatcgg agttgtggga ggcggcggcg gagtaggtct
1741 tggagtaggc ggaggcggag tgggaggcct gggaatgcag cacacacccc gaagcgattc
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1981 ctgtgagggc tgcaaggggt tctttcgacg cagcgttacg aagagcgccg tctactgctg
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2101 cctgaaaaag tgcctggccg tgggtatgcg gccggaatgc gtcgtcccgg agaaccaatg
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2221 gagctctcag catggcggca atggcagctt ggcctctggt ggcggccaag actttgttaa
2281 gaaggagatt cttgacctta tgacatgcga gccgccccag catgccacta ttccgctact
2341 acctgatgaa atattggcca agtgtcaagc gcgcaatata ccttccttaa cgtacaatca
2401 gttggccgtt atatacaagt taatttggta ccaggatggc tatgagcagc catctgaaga
2461 ggatctcagg cgtataatga gtcaacccga tgagaacgag agccaaacgg acgtcagctt
2521 tcggcatata accgagataa ccatactcac ggtccagttg attgttgagt ttgctaaagg
2581 tctaccagcg tttacaaaga taccccagga ggaccagatc acgttactaa aggcctgctc
2641 gtcggaggtg atgatgctgc gtatggcacg acgctatgac cacagctcgg actcaatatt
2701 cttcgcgaat aatagatcat atacgcggga ttcttacaaa atggccggaa tggctgataa
2761 cattgaagac ctgctgcatt tctgccgcca aatgttctcg atgaaggtgg acaacgtcga
2821 atacgcgctt ctcactgcca ttgtgatctt ctcggaccgg ccgggcctgg agaaggccca
2881 actagtcgaa gcgatccaga gctactacat cgacacgcta cgcatttata tactcaaccg
2941 ccactgcggc gactcaatga gcctcgtctt ctacgcaaag ctgctctcga tcctcaccga
3001 gctgcgtacg ctgggcaacc agaacgccga gatgtgtttc tcactaaagc tcaaaaaccg
3061 caaactgccc aagttcctcg aggagatctg ggacgttcat gccatcccgc catcggtcca
3121 gtcgcacctt cagattaccc aggaggagaa cgagcgtctc gagcgggctg agcgtatgcg
3181 ggcatcggtt gggggcgcca ttaccgccgg cattgattgc gactctgcct ccacttcggc
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3301 gacccagaac gattcccagc accagacaca gccgcagcta caacctcagc taccacctca
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3481 acctggttcc ttgtccgcgg tcagtacgag cagcgaatac atgggcggaa gtgcggccat
3541 aggacccatc acgccggcaa ccaccagcag tatcacggct gccgttaccg ctagctccac
3601 cacatcagcg gtaccgatgg gcaacggagt tggagtcggt gttggggtgg gcggcaacgt
3661 cagcatgtat gcgaacgccc agacggcgat ggccttgatg ggtgtagccc tgcattcgca
3721 ccaagagcag cttatcgggg gagtggcggt taagtcggag cactcgacga ctgcatagca
3781 ggcgcagagt cagctccacc aacatcacca ccacaacatc gacgtcctgc tggagtagaa
3841 agcgcagctg aacccacaca gacatagggg aaatggggaa gttctctcca gagagttcga
3901 gccgaactaa atagtaaaaa gtgaataatt aatggacaag cgtaaaatgc agttatttag
3961 tcttaagcct gcaaatatta cctattattc atacaaatta acatataata cagcctatta
4021 acaattacgc taaagcttaa ttgaaaaagc ttcaacaaca attggacaaa cgcgttgagg
4081 aaccgggaga aaatttaaga aaaaaaaaac cattgaaaat tatgaaattt agtatacatt
4141 tttttgggt ggatgtatgt cgcatcagac tcacgatcaa ttctcgaatt ttgttaacta
4201 aattgatcct ccaaactgca tgcgaaacag atcagaaaag agaacagaca gtagggcgtg
4261 aacagaggga agagagaaga gaataaagat tgtttatatt taaaaaatat ataaaataat
4321 aattactaac tctaaacgta atgaaagcaa ctgtataata tctaactata actataaatt
4381 cgtactgtag ggaagtgaga aaatctgtta aatgaaacaa aaataatgat aataacatta
4441 tcatccacca taattaaaat catttaaagt aattaaaaac aaaacacttt taaaacacgc
4501 aaaacttgga ctgattttat aaatattttt taatcataaa gaaaggcaac ctgaaaaaaa
4561 tattacaaaa acaaataaca acatatttta ttatgacacc cttatatgtt ttcaaaacga
4621 gaatttaaat tcttagattc ttataatttc atccaaaaat attagccagc aaaaaccttt
4681 attattggca ttgtttttag acatgtttc aaaaaaaact ttgatattga aactaaacaa
4741 aggataatga aatgaaagtg attggagtct tactcaaaaa ccaaaaggca tcaaaaggta
4801 ttaaattaaa aatataatct aatttcgagt tcaagaaaca ctttttggtg gaaaatagtt
4861 ttcaatcact ttgataaaaa ccacacaaat taataaatac atgcatacac caaaagactt

4921 caatatatat ttttaaaatt tacattgata attcgaaatt tgaataagaa tcacatccat
4981 ctaatttggc taaatcaaaa ttttatgaa agccacacaa aaaacgtgca aatttgatta
5041 ctttggcaat ttttatgtta tacaaaattt atgcaattga ttttcaaaat aatttttatt
5101 agattgtatt agtttcattt tgctttggga tgtacatttt aaataaattt tactttaaat
5161 tgttggcctt attttaactt aaatcaaatt tattctaatt ttagtaaaaa aaaatgtgtt
5221 taaaattgaa aataagaaca ctgtaaaata ttaataaaaa attaaagttt aaagtgattc
5281 ttttattatg taaaaagaag acaaaaaata tcttacgtag ctttctactt gaattgtgca
5341 atttttact tttactacta atcctaattt aaatataatt tacacacacg cctacacatc
5401 cagccacata ttttaattt taagtcaacc taatttataa atatgaattt gtataatgac
5461 gaactaaaat tagcatgaca tcatggacat acttggaaat aactctatca aacgagctaa
5521 atgcattgaa gaagaaaatt cttgttaaat atagtctgca cttcgacaaa cgaaaatcag
5581 tgaatt

35. SEQ ID NO: 35 Accession No. NM_165364 Drosophila melanogaster Hormone receptor-like in 39 CG8676-PD Hr39)

MPNMSSIKAEQQSGPLGGSSGYQVPVNMCTTTVANTTTTLGSSA
GGATGSRHNVSVTNIKCELDELPSPNGNMVPVIANYVHGSLRIPLSGHSNHRESDSEE
ELASIENLKVRRRTAADKNGPRPMSWEGELSDTEVNGGEELMEMEPTIKSEVVPAVAP
PQPVCALQPIKTELENIAGEMQIQEKCYPQSNTQHHAATKLKVAPTQSDPINLKFEPP
LGDNSPLLAARSKSSSGGHLPLPTNPSPDSAIHSVYTHSSPSQSPLTSRHAPYTPSLS
RNNSDASHSSCYSYSSEFSPTHSPIQARHAPPAGTLYGNHHGIYRQMKVEASSTVPSS
GQEAQNLSMDSASSNLDTVGLGSSHPASPAGISRQQLINSPCPICGDKISGFHYGIFS
CESCKGFFKRTVQNRKNYVCVRGGPCQVSISTRKKCPACRFEKCLQKGMKLEAIREDR
TRGGRSTYQCSYTLPNSMLSPLLSPDQAAAAAAAAAVASQQQPHQRLHQLNGFGGVPI
PCSTSLPASPSLAGTSVKSEEMAETGKQSLRTGSVPPLLQEIMDVEHLWQYTDAELAR
INQPLSAFASGSSSSSSSSGTSSGAHAQLTNPLLASAGLSSNGENANPDLIAHLCNVA
DHRLYKIVKWCKSLPLFKNISIDDQICLLINSWCELLLFSCCFRSIDTPGEIKMSQGR
KITLSQAKSNGLQTCIERMLNLTDHLRRLRVDRYEYVAMKVIVLLQSDTTELQEAVKV
RECQEKALQSLQAYTLAHYPDTPSKFGELLLRIPDLQRTCQLGKEMLTIKTRDGADFN

LLMELLRGEH

36. SEQ ID NO: 36 Accession No. NM_165364 Drosophila melanogaster Hormone receptor-like in 39 CG8676-PDHr39)

1 actaacaaaa caaacatttt gctacttcgt cgcaggcggg actgtgttgc gtcgtgtgat
61 cgctagagcg gttgtggaat cggattcgag cgcaaaacac cgttcatgct gtgagcgaaa
121 aagagtggta gcgcctacag tggcatatgt agttaaatcc gtgaataagt gaaaaatccg
181 atatttgtcg tgcaataatt tcctcgattg gcatcaagtg gcttccagtc gggtacatat
241 tgcacaagaa atgttatacg cataatgtgc acgcaaatta aacgaattct ctatgaaaat
301 gtgactagaa tgtgagtcga acaaaacgag taaaacgtga aatcccaact ggcttttggg
361 taacaaatct tatcaacaca gcaacggaaa tacattaaaa tcttgataga ctgagaaagg
421 gacaattgga atactttag ttatttttaa atgttttaca acacaatgga actgcatcaa
481 cgacacctct caaactttta caaattgcac aactgagaaa tagtctttga taaataaata
541 aaatataaga aatcgctact gaaacaagat gccaaacatg tccagcatca aagcggagca
601 gcaaagcggt cctcttggag gaagtagcgg ctatcaagta ccggtcaaca tgtgcaccac
661 cacagtcgcg aatacgacga ccactttggg aagctccgcc gggggagcca ctggctcccg
721 gcacaacgtc tccgtgacaa acatcaagtg cgaactagac gaactaccgt caccgaacgg
781 caacatggtg ccggttatcg caaactacgt tcacggtagc ttgcgcattc cactcagtgg
841 acattcaaat catagggagt ccgattcgga ggaggagctg gcaagtattg agaacttgaa
901 ggttcggcga aggacggcgg cggacaaaaa tggtcctcgt ccaatgtcct gggagggcga
961 gctgagcgat actgaggtca acgggggcga agagctgatg gaaatggagc caacaattaa
1021 gagtgaggtg gtccctgctg ttgcaccccc acaacccgtc tgcgcactac aaccgataaa
1081 aacagagcta gagaacattg caggcgagat gcagattcaa gagaagtgtt acccccagtc

1141 caacacacaa catcacgctg ccacaaaatt aaaagtggcc ccgacgcaaa gtgatccgat
1201 caatctcaag ttcgaaccgc ctctgggaga caattctccg ctactggctg cacgtagcaa
1261 gtccagcagt ggaggccacc taccactgcc aacgaatccc agtcccgact ccgccataca
1321 ttccgtctac acgcacagct ccccctcgca gtcgcctctg acgtcgcgcc acgcccccta
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1561 gccgtccagt gggcaggagg cgcagaacct gagtatggac tctgcctcta gcaatctgga
1621 tacagtgggc ttaggatctt cgcacccccgc atctccggcg ggcatatcac gtcagcagtt
1681 gatcaactcg ccctgcccca tctgcggtga caagatcagc ggatttcatt acgggatttt
1741 ctcctgcgag tcttgcaagg gcttcttcaa gcgcaccgtg caaaatcgca agaactacgt
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2221 ggatgtagag catctgtggc agtacaccga tgcagagctg gcccgcatca accaaccact
2281 gtccgcattc gcctctggca gctcttcgtc gtcgtcatcg tcaggtacat cctcaggcgc
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2401 tgccaatcct gatcttatcg ctcatctctg caacgtggct gatcaccgtc tttataaaat
2461 cgtcaaatgg tgcaagagct tgccgctttt taagaacatt tcgatcgatg accaaatctg
2521 cttgctcatt aactcgtggt gcgagctgtt gctcttctcc tgctgtttta gatcaattga
2581 tactcctgga gagattaaaa tgtcacaagg caggaagata accctatcgc aggccaaatc
2641 aaatggcttg cagacttgca ttgaacggat gctcaaccta acagatcacc tgaggcgatt
2701 gcgcgttgat cgctacgaat atgttgccat gaaagttatt gtgctgttgc agtcagatac
2761 gacagagtta caggaagcgg taaaggtgcg cgagtgtcag gaaaaagctt tgcagagctt
2821 gcaagcttac accctggcgc attatcctga cacgccatcc aagtttgggg agctttttgct
2881 acgcattcct gatttgcagc gaacgtgcca gcttggcaag gagatgttga cgatcaagac
2941 tcgcgatgga gctgatttca atttgctaat ggagcttttg cgcggagagc attgacaatt
3001 gataactaag acggaaatct tttaccattg gcaaaacaag tttcacatat ttagtattag
3061 atatatatat tctatagata agatccttac tgtaagttct gaaaacatgt gcctaaaaac
3121 caaagccacg atagcagtca catcaggccc actggtcgag attaaatcca agagcaagat
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3241 tgttgttgtc ggctaaagtt tcagtaagaa aagtatatat tgattttgct atttatacat
3301 atttgactta tgtatagtgt aaactaaagc acacatggaa aatgaaaaga ctaaacaaat
3361 ttatttaaag attactttta ctattataga aaaaggggaa aaataaaaaa cacaaaggca
3421 gagaagaaaa tttagttaca acaggtagcg acattttttat attttcttat ataaggaaat
3481 attcaatgta ttttaaatat aaagccaaac ccgatttggt ttgggaaaga gctactgaaa
3541 ttttgatat ctatatattc atcactagaa gacgaatgaa tgtatccaat gtttaaatgt
3601 tgtagcgttt agttttagtg caatttcaca catgtctaca tacatgaata ttcagcgaga
3661 tatgtttgca aactattata aagcaaaaga ccactcgaaa tcgccatcac tgggttggct
3721 aagactattc cagttatgct gtttgttgca taaaaaacca caactacgta catcaataaa
3781 atgtataatt ttttattgga gttttagatt tgtattaact tcttccttat aattacgatt
3841 attattatta ttactaattt tatgaatatt gtgtaacact gacttaaata gctgaaaaaa
3901 tcctgcaaca ggatttaaaa cacctgaata cacaaaacat tataacatga atacattttg
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4141 ccggaagtcc aagagatttg gctgaaagta tcgacaaatt ttcaacacat cgttcatgga
4201 tattgtgcta acactctcag tttgaaaatc attttctgtt aaactttcta tataataagt
4261 tctccattcg attttgtatt tacaatttgt ttctttaatt ttcctttatc agttgtatct
4321 atgaaacatg aggatctcag ttcatattga tcgtgttctt ctgccgtaca ccgcttctgt
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V. CLAIMS

What is claimed is:

1. A composition comprising an inhibitor of DHR96 activity.
2. A composition comprising an inhibitor of DHR96 activity and a pesticide.
3. An insect comprising a gene, wherein the gene comprises a mutation of the DHR96 gene.
4. A method of enhancing the effect a pesticide has on an insect comprising administering t o the insect an inhibitor of DHR96 activity.
5. A method of identifying a compound which will enhance the activity of a pesticide comprising incubating the compound with an insect, and assaying if the compound inhibits DHR96 activity.

VI. ABSTRACT OF THE DISCLOSURE

342. Disclosed are compositions and methods for modulating DHR96 activity and identifying molecules that modulate DHR96 activity.

DHR96 homologs in humans and *C. elegans*

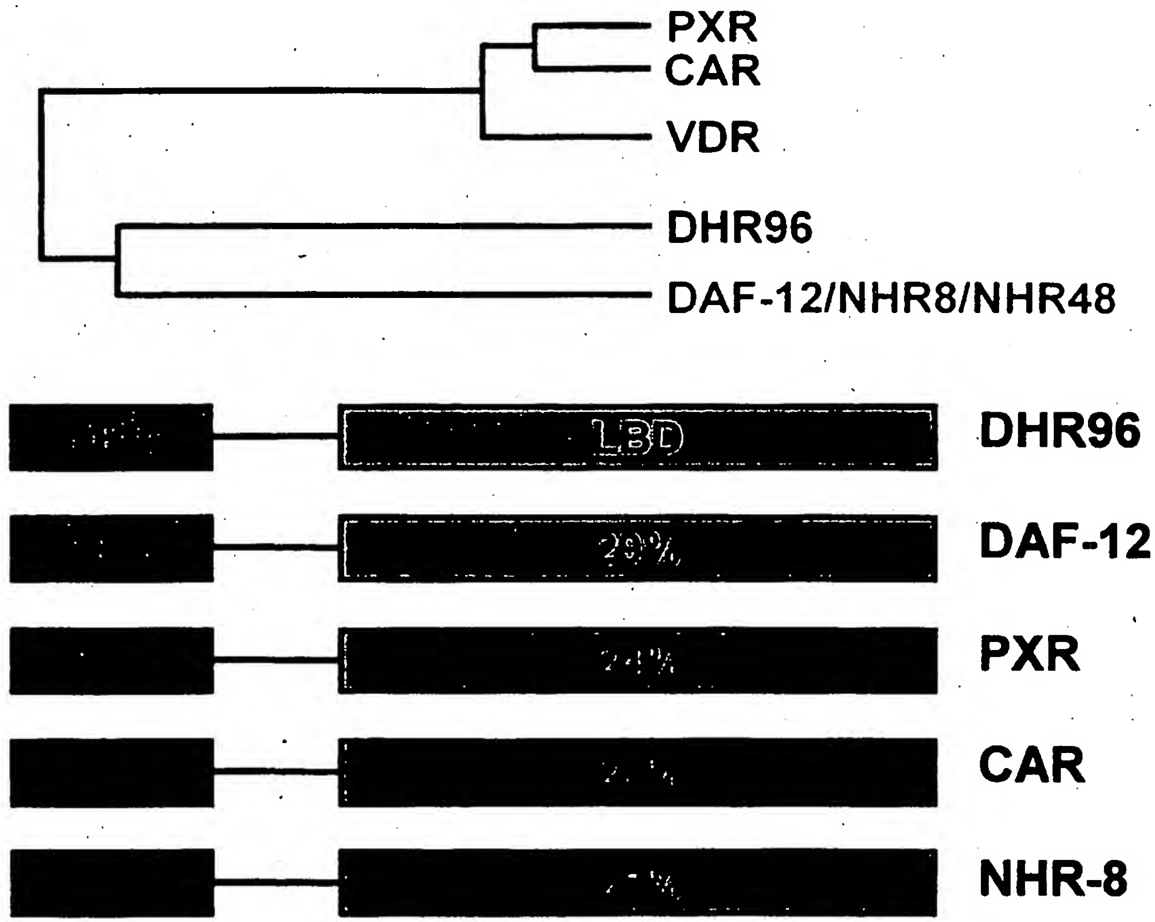
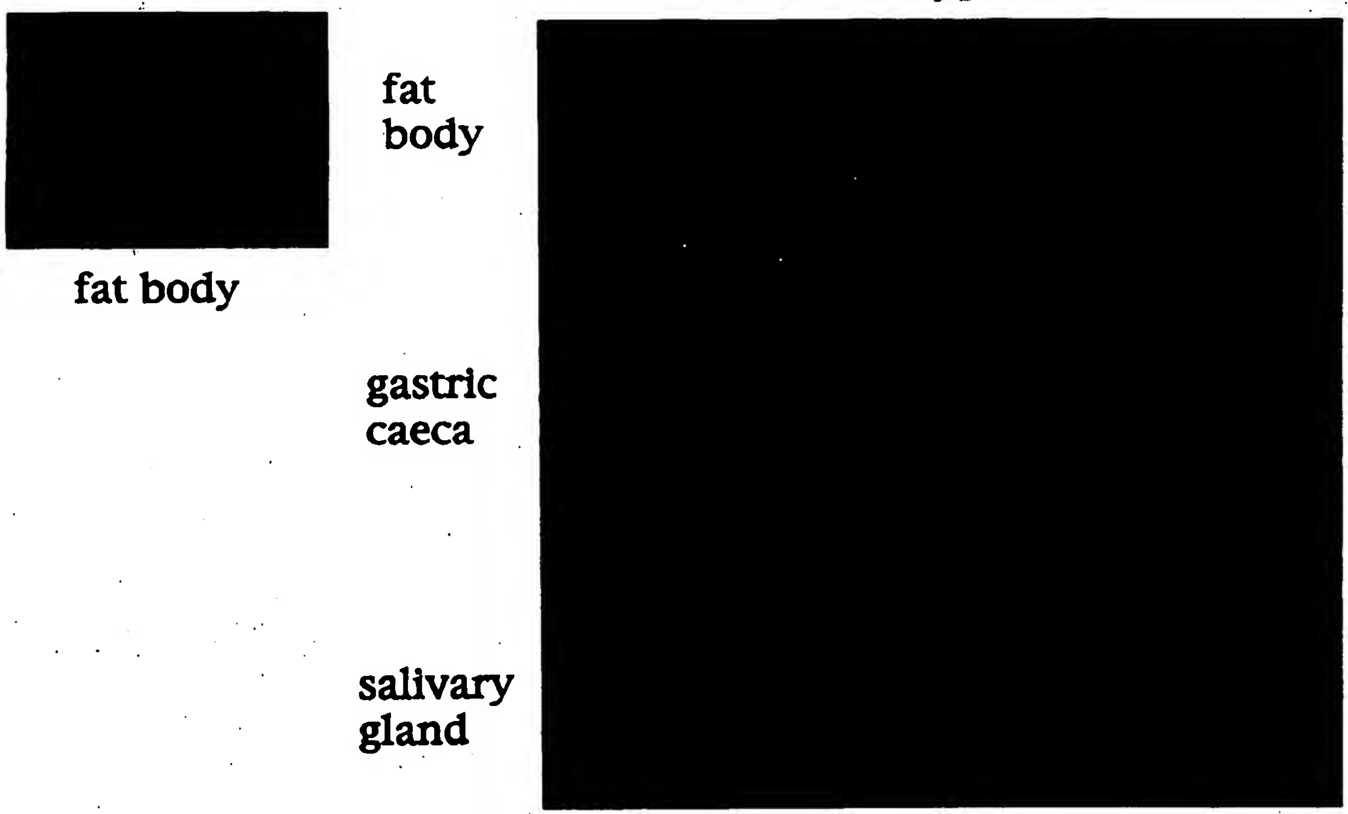


Figure 1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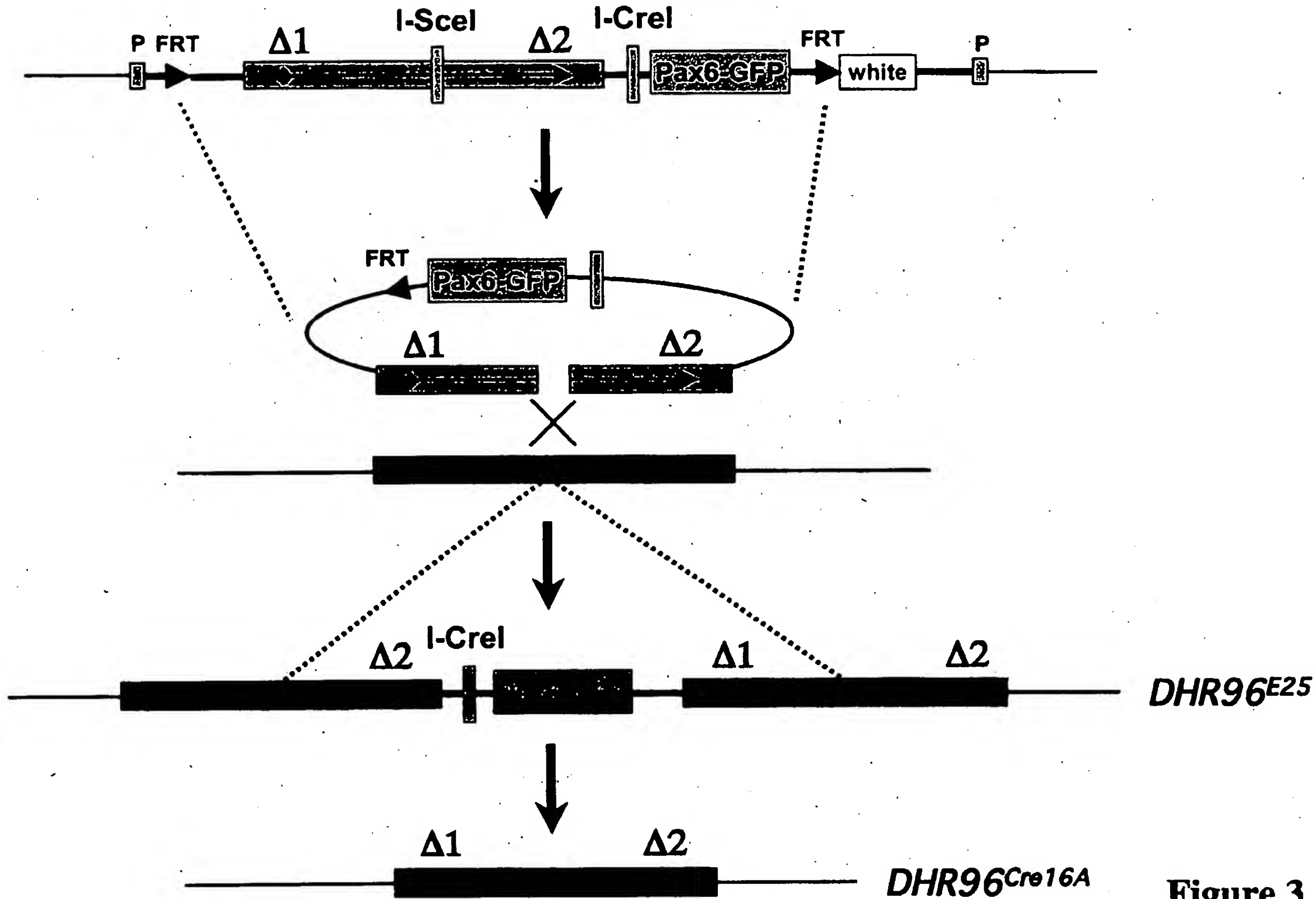
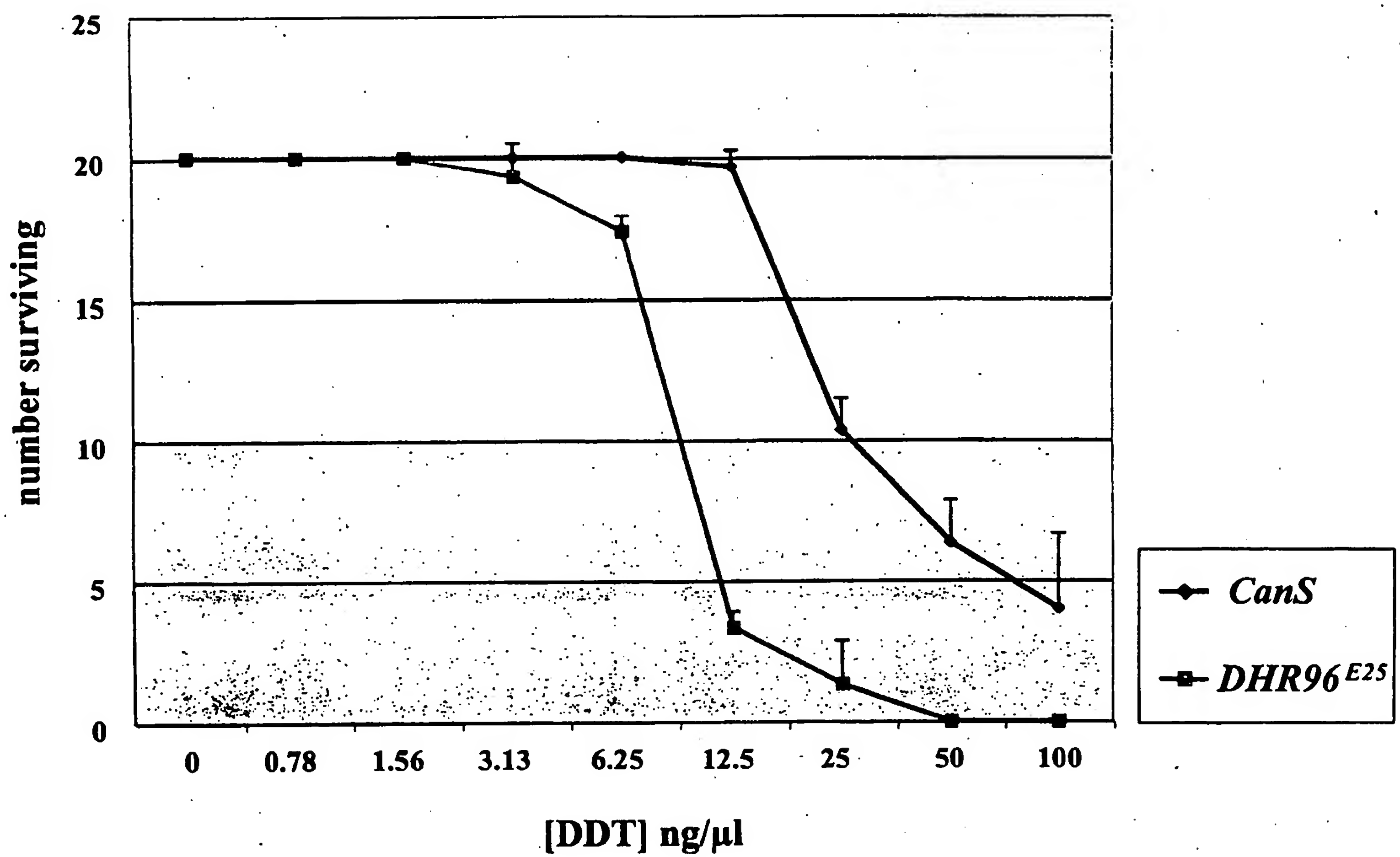
fat body
wild type
fat body
gastric caeca
salivary gland
Malpighian tubule
brain
imaginal disc

Figure 2



P FRT
Δ1
I-SceI
Δ2
I-CreI
Pax6-GFP
FRT
white
P
FRT
Pax6-GFP
Δ1
Δ2
Δ2
I-CreI
Δ1
Δ2
DHR96E25
Δ1
Δ2
DHR96Cre16A

Figure 3



number surviving
25
20
15
10
5
0
0
0.78
1.56
3.13
6.25
12.5
25
50
100
[DDT] ng/µl
CanS
DHR96 E25

Figure 4A

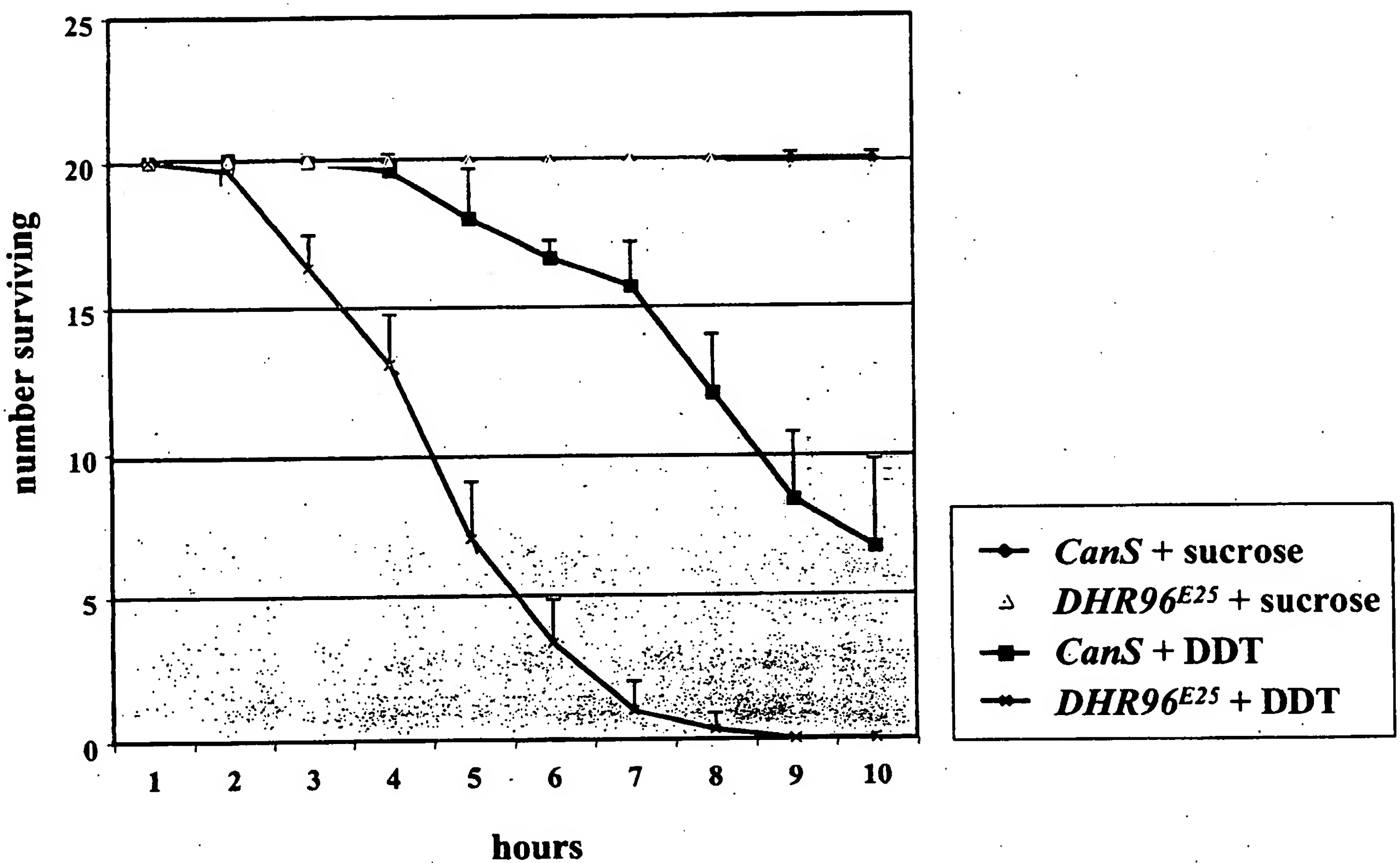

25
20
15
10
5
0
number surviving
1
2
3
4
5
6
7
8
9
10
hours
CanS + sucrose
DHR96E25 + sucrose
CanS + DDT
DHR96E25 + DDT

Figure 4B

```
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                                                              <----- R5
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                                                             <---------- R6
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                                  unique region
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Figure 5

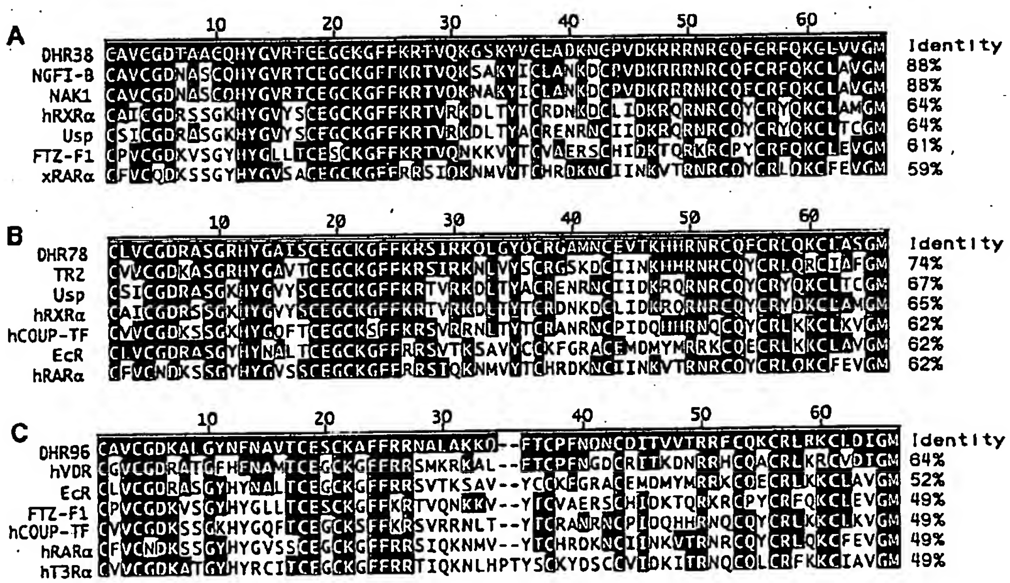

Figure 6

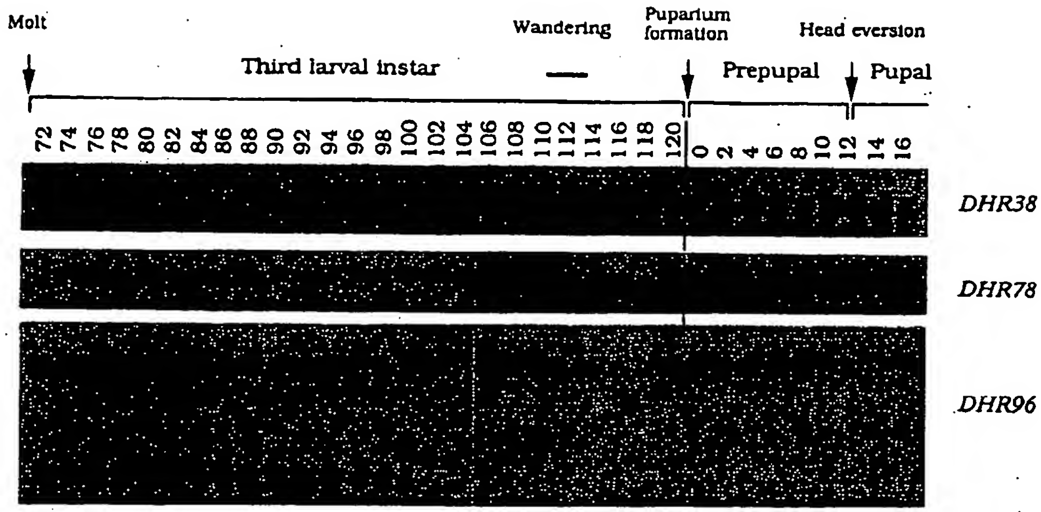

Figure 7

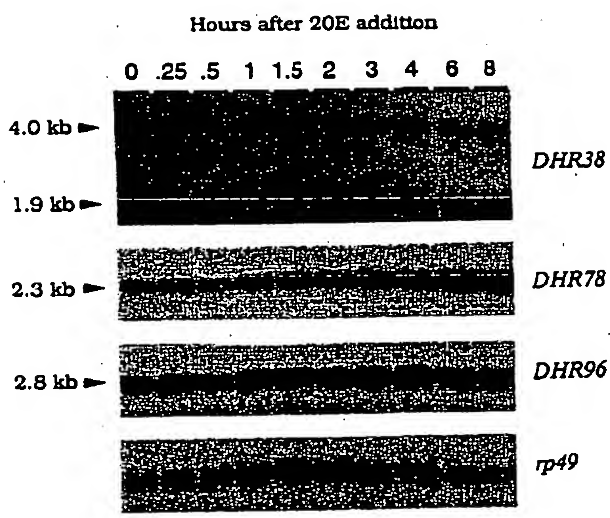

Figure 8

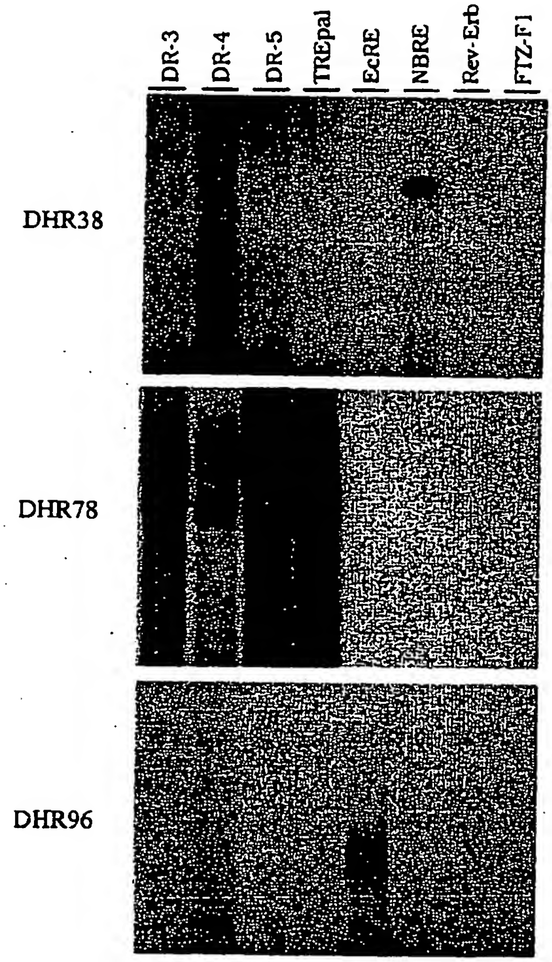

Figure 9